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Enantiomeric profiling of chiral drug biomarkers in wastewater with the usage of chiral liquid chromatography coupled with tandem mass spectrometry

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Abstract

This paper proposes a novel multi-residue enantioselective method utilising a CBH (cellobiohydrolase) column, for the analysis of 56 drug biomarkers in wastewater. These are: opioid analgesics, amphetamines, cocaine, heroin, stimulants, anaesthetics, sedatives, anxiolytics, designer drugs, phosphodiesterase-5 (PDE5) inhibitors, amphetamine and methamphetamine drug precursors. Satisfactory enantiomeric separation was obtained for 18 pairs of enantiomers including amphetamine, methamphetamine, MDMA (3,4-methylenedioxy-methamphetamine) and its metabolites HMA (4-hydroxy-3-methoxyamphetamine) and HMMA (4-hydroxy-3-methoxy-methamphetamine), PMA (para-methoxyamphetamine), MDA ((±)- 3,4-methylenedioxyamphetamine) and mephedrone. The method was applied in a one week monitoring study of a large wastewater treatment plant in the UK. Most target drugs were found at quantifiable concentrations in analysed samples. Enantiomeric profiling revealed that amphetamine, methamphetamine and MDMA were found enriched with *R*-(-)-enantiomers, probably due to their stereoselective metabolism favouring *S*-(+)-enantiomers. MDA was either enriched with *R*-(-)- or *S*-(+)-enantiomer indicating that its presence might be due to either abuse of racemic MDA or abuse of racemic MDMA respectively. Non-racemic enantiomeric fractions were also observed in the case of HMMA and mephedrone suggesting enantioselective metabolism. To the authors' knowledge, this is the first time chiral separation and wastewater profiling of mephedrone, PMA, MDMA and its metabolites HMA and HMMA have been reported.

Keywords: chiral drugs, enantiomers, chiral chromatography, wastewater, wastewater-based epidemiology, illicit drugs

1. Introduction

Wastewater-based epidemiology (WBE) has the potential to inform public health via the analysis of human urinary biomarkers in wastewater [1]. WBE is an emerging field but it has already found applications in verifying spatial and temporal community-wide illicit drug [2, 3], alcohol [4] or tobacco use [5].

An understanding of human pharmacokinetics and the selection of potential biomarkers informing public health is key to successful application of the WBE approach. As human pharmacokinetics shows stereoselectivity in the case of many chiral xenobiotics [6], chirality is also important to investigate in WBE.

In a recent study, the enantioselective separation of common illicit drugs revealed changes in enantiomeric composition of chiral drugs during wastewater treatment [7, 8]. In particular, it was demonstrated that the type of chiral drug, the treatment technology used in a wastewater treatment plant and the season affected the stereoselective enrichment or depletion of the enantiomeric composition of a drug. In another study, microbial metabolic processes were found to be responsible

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for stereoselective degradation of amphetamine-like compounds in river [9] and activated sludge microcosms [10].

Unfortunately in WBE, chiral analysis still has limited application despite its high potential in helping to understand for example: (i) the different route of synthesis of the drugs, (ii) the differentiation between the abuse and the licit use of drugs, (iii) the origin of a drug residue, differentiation between consumption and disposal of unused drugs and (iv) and the potency of the abused drug [11]. The concept of enantiomeric profiling in WBE has been applied for the first time by Kasprzyk-Hordern et al. (2012) [11]. In fact, from a study conducted in 7 WWTPs in England for 5 months, it was possible to conclude that MDMA was found in the influent wastewater samples due to its abuse rather than its direct disposal. Also, the presence of MDA was associated with abuse of MDMA and not abuse of MDA. In another study by Emke et al. (2014) [12], chiral analysis was key in confirming that unexpectedly high loads of MDMA observed in wastewater from one of Dutch cities were a result of dumping of MDMA from a local production facility during a police raid.

In order to undertake enantiomeric profiling of wastewater for chiral drug biomarkers, robust and multi-residue chiral analytical methods need to be developed. Until now, chiral LC-MS (liquid chromatography coupled with tandem mass spectrometry) methods were utilised in the investigation of a limited number of chiral drugs [11-13]. Therefore, chiral methods were used only as complementary tools alongside non-chiral LC-MS methods. Those approaches required an *ad hoc* sample preparation, which meant higher sample volume, more time consuming and less cost effective analysis.

This paper proposes, for the first time, a multi-residue method utilising a CBH (cellobiohydrolase) column for the analysis of 56 drug biomarkers at enantiomeric level, including satisfactory enantiomeric separations for 18 pairs of enantiomers. To the authors' knowledge, this method is the first to allow for:

(i) simultaneous and mutiresidue differentiation between the abuse and the licit use of drugs (e.g. in the case of amphetamine as illicit amphetamine, as opposed to prescribed licit amphetamine, is distributed as racemate),
(ii) verification of the origin of a drug residue (e.g. methamphetamine as chiral signature of methamphetamine is route of synthesis dependent),
(iii) differentiation between consumption and disposal of unused drugs (e.g. in the case of MDMA, fluoxetine and other targeted chiral illicit drugs. This is due to the fact that metabolic processes in humans are stereoselective and lead to changes of chiral signature of excreted drugs when compared to their unused counterparts)
(iv) verification of the potency of the abused drug (e.g. S-(+)- enantiomers of amphetamine and methamphetamine are known to be much more potent than R-(-) enantiomers of the same drugs).

The developed and validated method enabled the identification, detection and quantification of most targeted human biomarkers in wastewater. The method was applied in a one week monitoring study of a large wastewater treatment plant in the UK. Wastewater profiling of 56 biomarkers was undertaken. These are: opioid analgesics, amphetamines, cocaine, heroin, stimulants, anaesthetics, sedatives, anxiolytics, designer drugs, PDE5 inhibitors, amphetamine and methamphetamine drug precursors (Table 1). To the authors' knowledge, this is the first time chiral separation and then wastewater profiling of mephedrone, MDMA and its metabolites HMA, HMMA, PMA (para-methoxyamphetamine) using chiral CBH-HPLC-MS/MS method has been reported. The latter compound is a phenylisopropylamine with hallucinogenic properties, responsible, alongside N-monomethyl analogue (PMMA), for several deaths due to its abuse [14-16].

2. Experimental

2.1.Chemicals and materials

The following analytes were selected for the study (Table 1): opioid analgesics, amphetamines, cocaine, heroin, stimulants, anaesthetics, sedatives, anxiolytics, designer drugs, PDE5 inhibitors, amphetamine and methamphetamine drug precursors. Table S1 shows all target analytes, their CAS number, molecular formula, molecular weight, pK_a and supplier information.

The following deuterated analogues of target analytes were used as internal standards (IS): cocaine-d3, benzoylecgonine-d8, cocaethylene- d3, ecgonine methyl ester- d3, amphetamine-d5, methamphetamine-d5, phencyclidine-d5, mephedrone-d3, MDA-d5, MDMA-d5, MDEA-d5, cotinine-d3, EDDP-d3, heroin-d9, codeine-d6, oxycodone-d6, hydrocodone-d6, morphine-d6, morphine-3β-D-glucuronide-d3, methadone-d9, temazepam-d5, diazepam-d5, nordiazepam-d5, nitrazepam-d5, oxazepam-d4, lorazepam-d4, zopiclone-d4, ketamine-d4, norketamine-d4 and 1S,2R-(+)-ephedrine-d3.

The following analytes were used as racemates: (±)-mephedrone, (±)- 4-hydroxy-3-methoxymethamphetamine (HMMA), (±)- 3,4-methylenedioxymethamphetamine (MDMA), (±)- 4-hydroxy-3-methoxyamphetamine (HMA), (±)- methamphetamine, (±)- amphetamine, (±)- 3,4-methylenedioxyamphetamine (MDA), (±)- tramadol, (±)- desmethylvenlafaxine, (±)- venlafaxine, (±)- 3,4-methylenedioxy-N-ethyl-amphetamine (MDEA), (±)- ephedrine, (±)- pseudoephedrine, (±)-para-methoxyamphetamine (PMA), (±)- norephedrine, (±)- norfluoxetine, (±)- zopiclone, (±)-fluoxetine, (±)- 3,4-dihydroxymethamphetamine (DHMA), (±)- methadone, (±)- ketamine, (±)-norketamine, (±)- 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), (±)- lorazepam, (±)-temazepam, (±)- oxazepam. Enantiomerically pure standard solutions were used for the following analytes: 6-monoacetylmorphine with five defined stereocentres; oxycodone with four defined stereocentres, also known as (-)-oxycodone; morphine-3β-D-glucuronide with ten defined stereocentres; hydrocodone with four defined stereocentres; dihydromorphine with five defined stereocentres; codeine, also known as (-)-codeine with five defined stereocentres; morphine, also known as D-(-)-morphine with four defined stereocentres; normorphine with five defined stereocentres; heroin with five defined stereocentres; dihydrocodeine, also known as (-)-dihydrocodeine with five defined stereocentres; noroxycodone with four defined stereocentres; oxymorphone, also known as (-)-oxymorphone with four defined stereocentres; cocaethylene with four defined stereocentres; cocaine, also known as (-)-cocaine with four defined stereocentres; benzoylecgonine, also known as (-)-benzoylecgonine with four defined stereocentres and anhydroecgonine methyl ester (AEME) with two defined stereocentres.

All standards and internal standards were of the highest purity available (>97%). Stock and working solutions of standards were stored at -20° C. Methanol, acetonitrile and ammonium acetate were purchased from Sigma Aldrich, UK. Ultrapure water was obtained from PURELAB UHQ-PS Unit (Elga, UK). The deactivation of the glassware was carried out in order to prevent the adsorption of polar compounds to the hydroxyl sites on the glass surface. The process consisted of the following steps: rinsing of the glassware with 5% DMDCS once, with toluene twice and with methanol thrice.

2.2.Sample collection, storage and sample preparation

24h time-proportional (10 mL every 15 minutes) composite wastewater influent samples were collected in PTFE bottles from a local wastewater treatment plant. They were then transported to the laboratory in cool boxes packed with ice blocks and filtered through GF/F 0.7 µm glass fibre filter (Whatman, UK). 100 µL of a mixture of internal standard at concentration 1 mg L^{-1} were added to 100 mL of a wastewater sample to give final concentration of 1 µg L^{-1}. Stability of analytes in wastewater has been already investigated in our previous work [17, 18].

Solid phase extraction (SPE) was carried out using Oasis HLB cartridges (60 mg, Waters, UK) and the following procedure. The cartridges were conditioned with 2 mL of methanol followed by equilibration with 2 mL of ultrapure water at a rate of 3 mL min^{-1}. 100 mL of environmental sample (spiked with ISs at 1 µg L^{-1}) were passed through the HLB cartridge at a rate of 8 mL min^{-1}. The cartridges were then washed with 3 mL of ultrapure water at a rate of 3 mL min^{-1} and the analytes were eluted with 4 mL of methanol at a rate of 8 mL min^{-1} into 5 mL silanised glass tubes. The extract

was transferred to the TurboVap evaporator (Caliper, UK). After evaporation to dryness under nitrogen flow (5-10 psi) at 40°C the samples were reconstituted with 0.5 mL 1mM ammonium acetate/methanol 85:15 v/v and filtered through 0.2 µm PTFE filters (Whatman, Puradisc, 13mm). The filtered samples were transferred to polypropylene plastic vials bonded pre-slit PTFE/Silicone septa (Waters, UK) and then 20 µL were directly injected into a UHPLC-MS/MS system. Samples from monitoring campaign were prepared in duplicate and analysed twice.

2.3. Sample analysis with chiral liquid chromatography coupled with tandem mass spectrometry

Separation of all analytes was undertaken with Waters ACQUITY UPLC® system (Waters, Manchester, UK). Three chiral columns were evaluated in this study: (1) CHIRALPAK® CBH HPLC Column 5 µm particle size, L × I.D. 10 cm × 2.0 mm (Chiral Technologies, France) with a Chiral-CBH guard column 10 × 2.0 mm, 5 µm particle size (Chiral Technologies, France); (2) CHIROBIOTIC V column 5 µm particle size, L × I.D. 25 cm × 2.1 mm (Sigma Aldrich, UK) with a guard column 2 cm × 4.0 mm, 5 µm particle size (Sigma Aldrich, UK); (3) CHIROBIOTIC T column 5 µm particle size, L × I.D. 25 cm × 2.1 mm (Sigma Aldrich, UK) with a guard column 2 cm × 4.0 mm 5 µm particle size (Sigma Aldrich, UK).

ACQUITY UPLC™ autosampler was kept at 4°C, while the column temperature was set at 25°C. The injection volume of the sample was 20 µL. Several mobile phase compositions were tested (see Tables S2, S3 and S4 for details). Different flow rates were also trialled: 0.075 mL min^{-1} and 0.1 mL min^{-1}. The selected chiral column was the CHIRALPAK® CBH HPLC column. The chosen mobile phase used in the method was 1mM ammonium acetate/methanol 85:15 v/v at a 0.1 mL min^{-1} under isocratic conditions.

All analytes were identified and quantified using a triple quadrupole mass spectrometer (Xevo TQD, Waters, Manchester, UK) equipped with an electrospray ionisation source. Analyses were performed in positive mode with an optimised capillary voltage of 3 kV, source temperature of 150°C, desolvation temperature of 265°C and desolvation gas flow of 550 l h^{-1}. Nitrogen, supplied by a high purity nitrogen generator (Peak Scientific, UK), was used as a nebulising and desolvation gas. Argon (99.999%) was used as a collision gas. MassLynx 4.1 (Waters, UK) was used to control the Waters ACQUITY system and the Xevo TQD. Data processing was carried out on TargetLynx software (Waters, Manchester, UK).

The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode measuring the fragmentation of the protonated pseudo-molecular ions of each compound. The choice of fragmentation ion for each compound was based on the most intense signal. MRM transitions as well as cone voltages and collision energies were obtained after direct infusion of each standard at a concentration of 100 µg L^{-1} into the mass spectrometer. In the final stage of the method development, once CBH column was chosen, cone voltages and collision energies were optimised for the chosen MRM transitions through infusion of each standard at 100 µg L^{-1} combined with LC using 1mM ammonium acetate/methanol 85:15 v/v as mobile phase at 0.1 mL min^{-1} under isocratic conditions. Two or three MRM transitions were selected for each compound. The most abundant transition product ion was typically used for quantification with second and third transitions, for nearly all compounds, used for confirmation purposes. The MRM transitions of the studied compounds, cone voltages and collision energies are presented in Table 2.

Selection of internal standards (see Table 3) for those compounds for which deuterated or C13 analogues were not available commercially or in our laboratory was based on structural similarity and elution time to account for possible signal suppression/enhancement of studied analytes in ESI.

2.4.Method validation

The developed method was fully validated for wastewater samples. The following parameters were studied: instrumental and method limits of detection and quantification, linearity, precision and accuracy, ion suppression, resolution of enantiomers and enantiomeric fraction. Due to the potential

presence of target analytes in wastewater deuterated analogues of the targeted analytes were used as internal standards and to evaluate method performance.

The instrumental limit of detection (IDL) was determined at a concentration value giving a signal-to-noise ratio (S/N) ≥ 3 for all the MRM transitions selected for each substance. The method detection limit (MDL) was calculated using the following formula:

$$MDL = \frac{(IDL \times 100)}{\mathrm{Re}\ c \times CF} \quad (1)$$

where *IDL* is the instrumental limit of detection, *Rec* is the relative SPE recovery of the analyte in the matrix and *CF* is the SPE concentration factor.

The instrumental limit of quantification (IQL) was determined at the minimum concentration value giving S/N ≥ 10 for all the MRM transitions. The method quantification limit (MDL) was calculated using the following formula:

$$MQL = \frac{(IQL \times 100)}{\mathrm{Re}\ c \times CF} \quad (2)$$

where *IQL* is the instrumental limit of quantification, *Rec* is the relative SPE recovery of the analyte in the matrix and *CF* is the SPE concentration factor.

The linearity of the method was verified for each compound in the following range: IDL - 1000 µg L^{-1}. The individual calibrators were at a concentration of 1000, 800, 700, 600, 500, 400, 300, 200, 100, 50, 10, 5, 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, 0.005 and 0 µg L^{-1}.

For some compounds, especially human indicators, such as caffeine and creatinine, dilution integrity was considered as these substances are present at high concentrations in wastewater in respect to the illicit drugs concentration range. Dilution integrity was assessed through the analysis of two diluted samples 1:10 and 1:100 at the highest concentration in wastewater spiked with a mixture of ISs. If the compound could be quantified with a relative error within the 15% in relation to the nominal concentration, the dilution integrity was maintained.

Precision, expressed as relative standard deviation (RSD) of replicate analysis (n=4) at three different concentrations on the same day (intra-RSD%), was evaluated as:

(i) instrumental precision using standard solutions spiked in mobile phase at 10, 100 and 1000 µg L^{-1} for (non-chiral/not enantiomerically separated) analytes, or at 5, 50 and 500 µg L^{-1} for individual enantiomers (separated from racemic mixture);

(ii) method precision using standard solutions spiked in 100 mL of influent wastewater at 50, 500 and 5000 ng L^{-1} for (non-chiral/not enantiomerically separated) analytes, or at 25, 250 and 2500 ng L^{-1} for individual enantiomers (separated from racemic mixture). The extraction by SPE of these samples followed the same protocol described in 2.2.

Reproducibility (inter-day precision) of the method was determined by replicate measurements (n=3) of the same concentrations of analytes as in the case of intra-day precision on three different days in order to assess the inter-day instrumental precision and the inter-day method precision. Precision data were acceptable when the RSD% was less than 15% for all the concentrations investigated during the different days.

Accuracy of the method was expressed as percentage of closeness agreement between the mean of a set of analytical results and the theoretical value.

Carryover was studied by injecting a spiked sample at a concentration of 1000 µg L^{-1} followed by three blanks and it was considered insignificant if the concentration of the analyte was below the LOQ.

Ion suppression was calculated for each analyte as a percentage decrease in signal intensity in a sample matrix versus in mobile phase (free from analytes). Signal suppression was calculated using the following equation:

$$Signal \quad suppressio \quad n\,[\%] = \left(1 - \frac{Is - Io}{I_{MP}}\right) * 100 \tag{3}$$

where I_S was the analyte peak area in wastewater extract (0.5 mL) spiked after SPE extraction with 100 ng, I_0 was the analyte peak area in unspiked wastewater extract, I_{MP} was the analyte peak area in mobile phase (0.5 mL) spiked with 100 ng of each analyte.

Resolution of enantiomers of chiral drugs (R_s) was calculated using the following equation:

$$R_s = \frac{2\left(t_{rE2} - t_{rE1}\right)}{\left(w_{bE2} + w_{bE1}\right)} \tag{4}$$

where t_{rE1} and t_{rE2} are retention times of the first- and the second-eluted enantiomer respectively and w_{bE1}, w_{bE2} are widths of their responses at a baseline. $R_s \geq 1.2$ indicates full baseline resolution. $R_s = 1$ indicates 2% overlap which is deemed acceptable for quantification purposes.

Enantiomeric fraction (EF) was calculated using the following equation:

$$EF = \frac{(+)}{[(+) + (-)]} \tag{5}$$

where (+) is the concentration of (+)-enantiomer or first eluted enantiomer, and (-) is the concentration of (-)-enantiomer or second eluted enantiomer. EF equals 1 or 0 in the case of enantiomerically pure compound, and 0.5 in the case of a racemate. The assessment of the absolute configuration of the first eluted or second eluted enantiomer was achieved through the injection of an enantiomerically pure standard (when available).

Validation protocols were in agreement with European Guidelines concerning the performance of analytical methods and the interpretation of results [19]).

2.5.Quantification and quality controls

The identification criteria for each analyte were as follows [19]:

- %RSD of relative retention time (RRT) should not exceed ±2.5% when compared to RRT of standard solution.
- All selected MRM transitions need to be present.
- The maximum permitted tolerance for relative ion intensities of MRM transitions should not change more than ±20% for ions with relative intensities of >50%, ±25% for ions with relative intensities between 20% and 50%, 30% for ions with relative intensities between 10% and 20% and ±50% for ions with relative intensities less than 10%.

Quality controls at 10, 100 and 1000 µg L^{-1} were also prepared and injected on regular basis to maintain instrument's performance.

3. Results and Discussion

3.1.Choice of biomarkers

Fifty six compounds were selected and targeted as potential human biomarkers of drug consumption. These are: opioid analgesics, amphetamines, cocaine, heroin, stimulants, anaesthetics, sedatives, anxiolytics, designer drugs, PDE5 inhibitors, amphetamine and methamphetamine drug precursors (Table 1). Multiple human urine indicators, such as creatinine, caffeine, nicotine, 1,7-dimethylxanthine, cotinine, were also targeted as indicators of population size served by a wastewater treatment plant in question.

The selection process of target drug biomarkers included the investigation of: (i) classical drugs of abuse, with good literature based evidence of their detection and quantification in wastewater; (ii) new emerging drugs of abuse for further study, even if prevalence data and stability data in wastewater are not well documented, and (iii) substances with abuse potential.

Cocaine, benzoylecgonine, anhydroecgonine methylester and cocaethylene were selected as biomarkers of cocaine abuse. Indeed, anhydroecgonine methylester, ethylecgonindine and ecgonidine were identified as suitable indicators of crack cocaine [20]. Moreover, cocaethylene was chosen as biomarker of co-administration of cocaine and ethanol [21]. Ecgonidine and norcocaine were not included in this study as they were not detected in a previous UK study by Baker et al. (2014). Furthermore, cuscohygrine, a marker of coca chewing [22], was also included in the method in order to distinguish between chewing of cocoa leaves (the "coqueo", a practise well-known in South America) and illegal abuse of cocaine. To the authors' knowledge, no investigation of cuscohygrine and hygrine (cocoa chewing markers) has been undertaken to date. It is however worth mentioning that the practise of chewing cocaine is a non-European habit. Cuscohygrine was included in the method development but not in the method validation due to low sensitivity and poor chromatography (results are included in the supplementary data).

3.2. Method development for the detection of illicit/licit abused drugs in wastewater

3.2.1. Chiral-CBH column

The CHIRAL-CBH column contains a protein cellobiohydrolase (CBH) as the chiral selector which is immobilised onto spherical 5 μm silica particles. The protein has a molecular weight of 60,000–70,000 and an isoelectric point of 3.9. The chiral recognition site is 4Å×7Å×40 Å-long tunnel in the core of the protein. The tunnel contains seven acidic amino acid residues, four tryptofan residues and also tyrosine, serine, threonine, arginine and histidine. The mechanism of retention of analytes in CHIRAL-CBH column can therefore involve a combination of ion exchange, hydrogen bonding and hydrophobic interactions. The enantioselectivity of the retention is regulated by the pH of mobile phase, the nature and concentration of the organic modifier and the aqueous buffer [7]. Therefore, in order to achieve the best chiral recognition of target chiral analytes within one analytical run, the following parameters were investigated in this study: type and concentration of organic modifier (acetonitrile, methanol an isopropanol) in aqueous mobile phase and concentration of ammonium acetate.

In order to undertake quantitative measurements at enantiomeric level we aimed at obtaining enantiomeric resolution with maximum 2% overlap for each pair of enantiomers ($R_s \geq 1$). Our study revealed that $R_s \geq 1$ was achieved only in the case of 3 compounds (HMA, fluoxetine and zopiclone) in 1mM ammonium acetate/acetonitrile 9:1, 7 compounds in 1mM ammonium acetate/isopropanol 9:1 and >10 compounds in 1mM ammonium acetate/methanol 8.5:1.5 (Figures S1-3). A comparison of mobile phases with the same water content revealed that the separation selectivity differed for protic and aprotic solvents. Acetonitrile (an aprotic solvent) did not provide an adequate separation selectivity as opposed to protic solvents such as methanol and isopropanol (fluoxetine was an exception). Moreover, better separation selectivity was observed for more polar methanol than isopropanol. Furthermore, the water content in mobile phases containing isopropanol or methanol had an impact on enantioselectivity in the case of most of the studied analytes. In fact, lower water content provided higher resolution of enantiomers.

Furthermore, different organic content in aqueous mobile phases affected retention times of many compounds as shown in Figure S4. A linear relationship between retention times and the methanol content with different concentrations of ammonium acetate was observed. Indeed, retention times decreased with higher concentration of ammonium acetate. Furthermore, they decreased with a higher concentration of methanol. In contrast, retention times increased with an increase of concentration of isopropanol (Figure S5).

The salt concentration plays a key role in controlling the pH of mobile phase, ionisation of analytes and resulting interactions between analytes and the stationary phase. In this study, ammonium acetate was used. The amphetamine-like compounds (except for PMA) showed higher resolution with lower concentration of ammonium acetate in the mobile phase. However, this trend was not observed in the case of cyclopyrrolone zopiclone and the substituted cyclohexanone norketamine (Figure S3).

3.2.2. Chirobiotic V and T

Chirobiotic V and T, two chiral columns having macrocyclic antibiotics as chiral selectors, were also tested. In reversed-phase conditions, not only cationic and anionic interactions are possible by changing pH of the mobile phase but also inclusion of the pocket and hydrogen bonding are favoured. In polar organic mode, other interactions are involved, such as dipole stacking and π-π complexation.

A comparison between CBH and Chirobiotic V columns was performed by Bagnall et al. 2012 [23]. Due to the utilisation of a combination of ion exchange, hydrogen bonding and hydrophobic interactions, CBH column was more selective in providing better enantiomeric resolution (i.e. $R_{s\ MDMA\ CBH}$ 1.9 > $R_{s\ MDMA\ CBV}$ 1.0) than Chirobiotic V. In general, CBH column provided better results in terms of separation and resolution of amphetamine-like compounds when compared to Chirobiotic V.

Experiments carried out with Chirobiotic T column showed higher enantioselectivity ($R_s \geq 2.2$) for benzodiazepines only, a class of compounds not enantiomerically resolved using CBH column (Figure S5). It is worth emphasising that polar organic mobile phases, containing only methanol and 99% methanol/0.005% FA/1mM ammonium acetate, provided the best chiral recognition for most of the chiral benzodiazepines. Furthermore, mobile phases containing comparable quantities of an acid as a mobile phase additive provided better chiral recognition at lower concentrations of ammonium acetate (Figure S6).

After taking above results into consideration, the best chiral recognition for the widest group of analytes, combined with acceptable retention times, was achieved with the CBH column and a mobile phase composed of 1mM ammonium acetate/methanol 85:15 (pH 6.4).

3.3.Method validation for the detection of illicit/licit abused drugs

3.3.1 Solid phase extraction

Oasis HLB cartridges are the sorbents of choice when utilising chiral separations with the CBH column. Relative recoveries data are reported in Table 4. Recoveries were high (on average > 90%) for all analysed compounds.

3.3.2. Instrumental and method validation parameters

Figure 1 shows mass chromatograms of MRM 1 transitions used for quantification purposes, for each investigated analyte of a spiked influent wastewater sample at a concentration of 500 ng L^{-1}. The developed method allowed for identification and quantification of all studied analytes with satisfactory sensitivity and specificity.

Concentrations of compounds were calculated using the standard calibration curves which were developed using a detector response defined as the ratio of the peak ion (the specific product ion of the highest intensity, MRM1) to the base peak ion of the internal standard. The mean correlation coefficients (R^2) of the calibration curves were on average > 0.997 for the investigated compounds (Table 5). The linearity ranges varied for different analytes. Most analytes showed linearity from 0.25 µg L^{-1} up to 500 or 1000 µg L^{-1} (for single enantiomer or racemate respectively). Opioids, DHMA, lorazepam, creatinine and 1,7-dimethylxanthine showed very good linearity in the range: 1 µg L^{-1} - 500 or 1000 µg L^{-1} (for single enantiomer or racemate respectively). Amphetamine-like compounds gave linearity from 0.125 µg L^{-1} to 500 or 1000 µg L^{-1} (for single enantiomer or racemate respectively) showing a high level of performance of the CBH column for these compounds. Cocaine and its

metabolites responded with a linearity range of 0.01 µg L^{-1} - 500 or 1000 µg L^{-1} (for single enantiomer or racemate respectively). In the case of compounds present in wastewater at high concentrations exceeding accepted linearity ranges, dilution (1:10 or 1:100) of samples was utilised. It was maintained with a relative error <15%.

Good enantiomeric resolution (R_s ≥1.0, allowing for quantification of individual enantiomers) was obtained for most analytes (Table 6). The following analytes: MDEA, HMMA, tramadol, fluoxetine, , ephedrine, norephedrine and desmethylvenlafaxine showed lower enantiomeric resolution and therefore results for single enantiomers of these compounds should be considered on a semi-quantitative basis.

Enantiomeric fractions for those analytes which were injected as racemates, were on average 0.49 and were reproducible across different concentration ranges (Table 6).

The instrumental limits of detection and quantification ranged from 0.005 to 10 µg L^{-1} and from 0.05 to 50 µg L^{-1} respectively (Table 5). The method limits of detection and quantification ranged from 0.03 to 61 ng L^{-1} and from 0.13 to 320.87 ng L^{-1} (Table 5). The instrumental and method precision was on average <5% and <10% respectively (Tables 7 and S5). Ion suppression studies showed how the presence of the internal standard deuterates compensated the ion suppression in the matrix, even for those compounds that had not its corresponding deuterated analogue. (Table S6).

3.3.3. Analysis of wastewater samples

The developed and validated method was applied in a one-week monitoring campaign of a wastewater treatment plant serving a large city in the UK. The results are provided in Table 8. Most target drugs were found at quantifiable concentrations in analysed samples. The results for several drugs such as cocaine and MDMA and their metabolites show a clear trend of increased concentration during weekends. Other target drugs showed constant concentrations across the sampling week. These are for example: morphine, ketamine, benzylpiperazine, dihydrocodeine, methadone, amphetamine and methamphetamine. These results will be used in further study to estimate drug use via wastewater-based epidemiology. It is worth noting that despite suspected high usage of zopiclone, fluoxetine and norfluoxetine, these drugs were not detected in analysed wastewater samples. This is probably because of relatively high MDL values for zopiclone, fluoxetine and its metabolite norfluoxetine in the developed method.

Amphetamine, methamphetamine and MDMA were found enriched with *R*-(-)-enantiomers, probably due to their stereoselective metabolism favouring *S*-(+)-enantiomers. MDA was either enriched with *R*-(-)- or *S*-(+)-enantiomer indicating that its presence might be due to either abuse of racemic MDA (excess of *R*-(-)-enantiomer should be observed if administered as racemate) or abuse of racemic MDMA (excess of *S*-(+)-enantiomer should be observed). As MDA is a minor and not exclusive metabolite of MDMA, other metabolites (HMMA, HMA, and DHMA) were targeted for the first time in wastewater. The trend observed for HMMA in terms of concentration was similar to the parent drug MDMA, whilst for HMA and DHMA the trends were not "superimposable" to that one of MDMA. Among the metabolites of MDMA investigated, this was the first time that the enantiomeric profiling of HMA and HMMA was studied in wastewater. In the developed method, the enantiomers of DHMA were not separated so evaluation of its enantiomeric profiling was not possible. Significant changes in enantiomeric fractions (between 0.40 and 0.58) were noticed in the case of HMMA suggesting enantioselective metabolism. The enantiomeric profiling of PMA was not undertaken as PMA was not detected in wastewater. Even though PMA is a minor metabolite of PMMA, as reported by Lin et al.2007, most PMMA is excreted unchanged in the urine. So, PMA could be a suitable biomarker only for PMA intake [24].

Temporal changes in mephedrone concentrations were observed with noticeable increase of mephedrone levels during weekends. This is the first time mephedrone was detected and quantified in wastewater in the UK. Mephedrone was also found to be enriched with E1 enantiomer, which

suggests enantioselective metabolism in humans. Further work is needed to support the above hypothesis.

Conclusions

Understanding patterns of drug use is of key importance in public health monitoring. WBE, a new non-intrusive tool, provides significant advances in the field. It allows for multiple temporal and spatial drug use estimates in near-real time. Enantiomeric profiling provides a new dimension to WBE as it can help with the verification of the origin of drug residue, potency of abused drug and its synthetic route. To aid enantiomeric profiling in WBE, a new analytical method utilising a CBH column and liquid chromatography coupled with tandem mass spectrometry was developed. The method showed very good performance: >90% SPE recoveries, very good sensitivity (MDLs and MQLs at ppt levels), high linearity range and method precision <10%. The method allowed for the analysis of 56 drug biomarkers in wastewater. These are: opioid analgesics, amphetamines, cocaine, heroin, stimulants, anaesthetics, sedatives, anxiolytics, designer drugs, PDE5 inhibitors, amphetamine and methamphetamine drug precursors. Satisfactory enantiomeric separation was obtained for 18 pairs of enantiomers including amphetamine, methamphetamine, MDMA and its metabolites HMA and HMMA, PMA, MDA and mephedrone. The method was applied in a one week monitoring study of a large wastewater treatment plant in the UK. Most target drugs were found at quantifiable concentrations in analysed samples. The results for several drugs such as cocaine and MDMA and their metabolites showed a clear trend of increased concentrations during weekend. Enantiomeric profiling revealed that amphetamine, methamphetamine and MDMA were found enriched with *R*-(-)-enantiomers, probably due to their stereoselective metabolism favouring *S*-(+)-enantiomers. MDA was either enriched with *R*-(-)- or *S*-(+)-enantiomer indicating that its presence might be due to either abuse of racemic MDA or abuse of racemic MDMA. Non-racemic enantiomeric fractions were also observed in the case of HMMA and mephedrone suggesting enantioselective metabolism. To the authors' knowledge, this is the first time chiral separation and wastewater profiling of mephedrone, PMA, MDMA and its metabolites HMA and HMMA is reported.

Supplementary Data

Supplementary material contains the following:

Table S1 Selected analytes and their properties.

Table S2 Studied mobile phase compositions with CHIRALPAK® CBH HPLC.

Table S3 Studied mobile phase compositions with CHIROBIOTIC V.

Table S4 Studied mobile phase compositions with CHIROBIOTIC T.

Table S5 Validation parameters -instrumental precision.

Table S6 Validation parameters- ion suppression.

Figure S1 CBH column - enantiomeric resolution of studied analytes in a mobile phase containing acetonitrile as organic modifier (mobile phase composition: 1mM ammonium acetate/acetonitrile 9:1).

Figure S2 CBH column - enantiomeric resolution of studied analytes in a mobile phase containing isopropanol as organic modifier (mobile phase composition: (a) 1mM ammonium acetate/isopropanol 9:1 and (b) 1mM ammonium acetate/isopropanol 9.5:0.5).

Figure S3 CBH column - enantiomeric resolution of studied analytes in mobile phases containing: (a) 1 mM ammonium acetate/methanol 9.5:0.5, (b) 1 mM ammonium acetate/methanol 9:1, (c) 2.5 mM ammonium acetate/methanol 9:1, (d) 5 mM ammonium acetate /methanol 9:1 and (e) 10 mM ammonium acetate /methanol 9:1.

Figure S4 CBH column - Impact of different percentanges of modifiers on retention time of analytes.

Figure S5 Chirobiotic T column - overview of the separation for the targeted analytes

Figure S6 Chirobiotic T column - separation of oxazepam and lorazepam.

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Figure 1 Chromatograms of the quantification MRM transition for each investigated analyte of a spiked influent wastewater sample at a concentration of 500 ng $L^{-1}$with CBH column

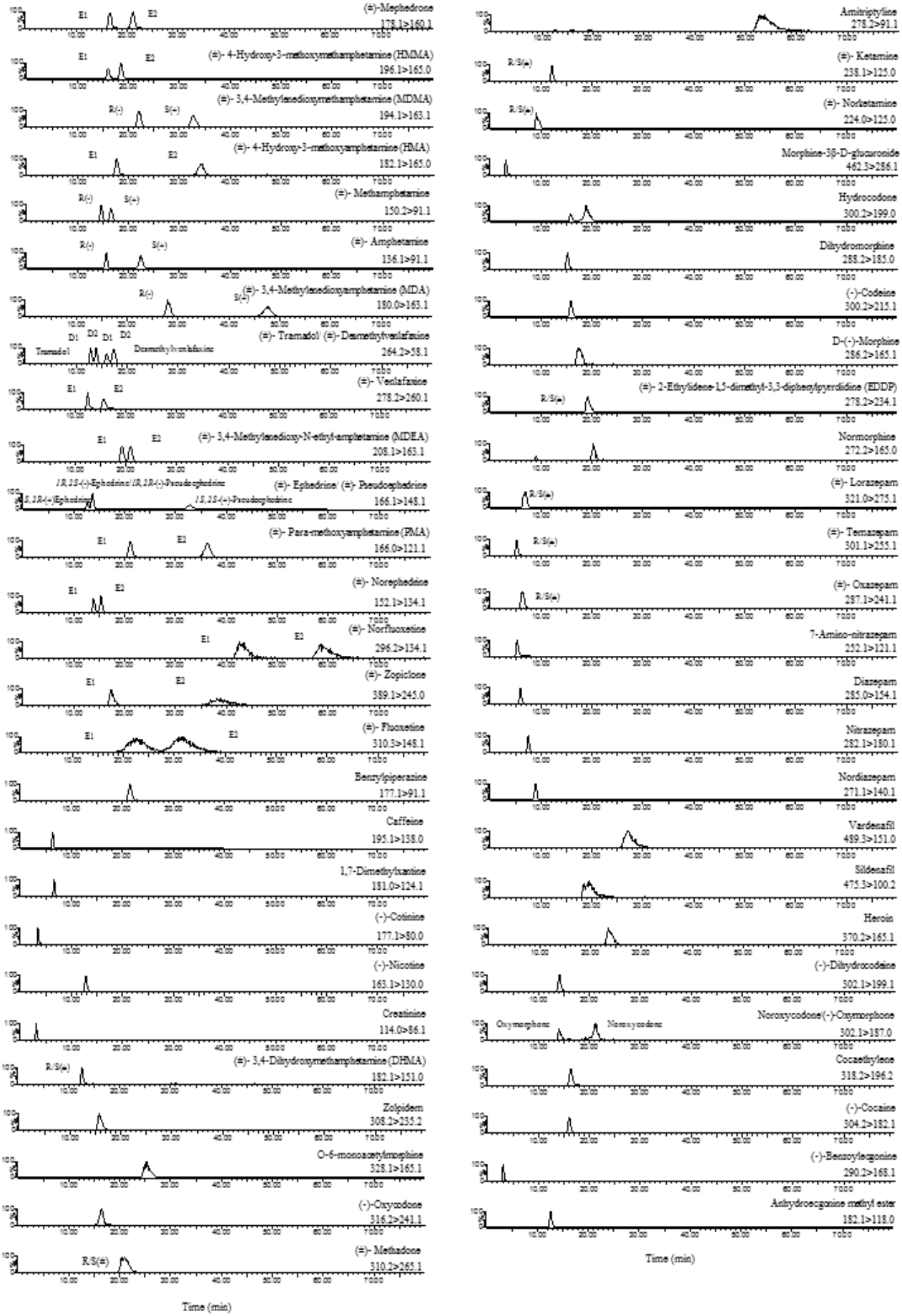


Table 1 Selected chiral drug biomarkers and their pharmacokinetic data

Group	Drug	Metabolite	Excretion	Source of excretion (range)
Stimulants	Cocaine	Cocaine	1.0-9.0%, 7.5%	[25]; [26]
		Benzoylecgonine	32.5% (nasal)	[27]
		Anhydroecgonine methyl ester (AEME)	0.7%	[28]
	Cocaine and alcohol	Cocaethylene		Drugbank [29], [28]
Stimulants	Amphetamine	Amphetamine	30.0% in neutral condition of pH, up to 74.0% in acidic and 1.0% in alkaline urines	[25]
		Norephedrine	2.0% in neutral condition of pH	[25]
Stimulants	Methamphetamine	Methamphetamine	43.0% at pH range between 6 and 8, up to 76.0% in acidic and 2.0% in alkaline urines	[25]
		Amphetamine	4.0-7.0% at pH range between 6 and 8	[25]
Stimulants	Mephedrone	Mephedrone	Unknown	
Hallocinogens	MDA	MDA	Unchanged (overdose case)	[30]
Hallocinogens	MDMA	MDMA	15.0%	[31]
		MDA	1.5%	[31]
		DHMA	minor	[32]
		HMMA	20.0%	[31]
		HMA	1.0%	[31]
Hallocinogens	MDEA	MDEA	19.0%	[25]
		MDA	28.0%	[25]
Opioids	Diamorphine	Diamorphine	0.1%	[33]
		Morphine derivative (O-6-MAM)	50.0-60.0%	[33]
Opioids	Morphine	Morphine	10.0%	[25]
		Morphine-3-glucuronide	75.0%	[25] [34]
		Hydromorphone (not targeted)	Trace	[34]
		Normorphine	Not found	(Doris Clouet 2012)
Dissociative agent	Ketamine	Ketamine	2.3%	[25]
		Norketamine	1.6%	[25]
Stimulants	Benzylpiperazine	Benzylpiperazine	3.0-6.0%	[35]
Benzodiazepines	Temazepam	Temazepam	1.5%+73.0% as conjugated	[25]
		Oxazepam	1.0%+5.8% as conjugated	[25]
Benzodiazepines	Diazepam	Diazepam	Trace	[25]
		Oxazepam gluc	33.0%	[25]
		Temazepam	6.0%	[25]
		Nordiazepam	Trace	(Steven B. Karch, 2007)
Benzodiazepines	Nitrazepam	Nitrazepam	trace, 1.0% (in the 7 day urine)	[25]
		7-amino-nitrazepam	31.0% (in the 7 day urine)	[25]

Benzodiazepines	Oxazepam	Oxazepam	trace+61.0% as glucuronide	[25]
Benzodiazepines	Lorazepam	Lorazepam	trace as unchanged + 75.0% lorazepam glucuronide	[25]
Population biomarkers	Caffeine	Caffeine	0.7-0.9%	
		1,7-dimethylxanthine	14.0%	[36]
Population biomarkers	Nicotine	Nicotine	13.0%, 5.0%	[5], [25]
		Cotinine	30.0%, 10.0%	[5], [25]
Population biomarkers	Creatinine			
Opioids	Codeine	Codeine	10.0%, 32.0-46.0% as glucuronide	Drugbank [29], [34]
		Morphine	5.0-13.0%	[34]
Opioids	Oxycodone	Oxycodone	13.0-19.0%+7.0-29.0% as conjugated	[25]
		Oxymorphone	13.0-14.0% as conjugated	[25]
		Noroxycodone	Trace	[25]
Opioids	Hydrocodone	Hydrocodone		
		Hydromorphone (not targeted)	5.0%	[34]
Opioids	Dihydrocodeine	Dihydrocodeine	31.0%, 28.0% as conjugated	[25]
		Dihydromorphine	8.4% as conjugated	[25]
Opioids	Methadone	Methadone	27.5 (5-50)	[30]
		EDDP	3.0-25.0%	[25]
Antidepressants	Venlafaxine	Venlafaxine	5.0%	[25]
		O-desmethylvenlafaxine	29.0-48.0%	[25]
Phosphodiesterase Type 5 Inhibitor	Vardenafil	Vardenafil	<10.0%	(Thomas L. Lemke, David A. Williams 2012)
Precursors	Ephedrine	Ephedrine	70.0-80.0%	[25]
		Norephedrine	4.0%	[25]
	Pseudoephedrine	Pseudoephedrine	88.0%	[25]
Stimulants	PMA			
Opioids	Tramadol	Tramadol	29.0%	[25]
		O-desmethyltramadol	20.0% as free and conjugated	[25]
Z-drugs	Zolpidem	Zolpidem	Nd	[25]
Antidepressants	Amitriptyline	Amitriptyline	Trace	Drugbank [29]
Phosphodiesterase Type 5 Inhibitor	Sildenafil	Sildenafil	13.0%	[25]
Z-drugs	Zopiclone	Zopiclone	4.5	[25]
Antidepressants	Fluoxetine	Fluoxetine	2.5-5.0%	[37]
		Norfluoxetine	10.0%	[37]

Table 2 MRM transitions selected for studied analytes

Compound	CV/CE[a]	MRM1 (quantification)	CV/CE[a]	MRM2 (confirmation)	CV/CE[a]	MRM3 (confirmation)	MRM1/MRM2 ratio ± SD	MRM1/MRM3 ratio ± SD	Internal standard
Cocaine	40/20	304.2 > 182.1	40/31	304.2 > 82.1	-	-	2.8 ± 0.2	-	Cocaine-D3
Benzoylecgonine	38/19	290.2 > 168.1	38/30	290.2 > 105.1	-	-	1.9 ± 0.2	-	Benzoylecgonine-D8
Cocaethylene	38/20	318.2 > 196.2	38/30	318.2 > 82.1	-	-	1.9 ± 0.1	-	Cocaethylene-D3
Anhydroecgonine methyl ester (AEME)	39/23	182.1 > 118.0	39/21	182.1 > 122.1	-	-	1.2 ± 0.1	-	Cocaine-D3
Amphetamine	18/16	136.16 > 91.1	18/8	136.16 > 119.1	-	-	1.2 ± 0.1	-	Amphetamine-D5
Methamphetamine	24/19	150.2 > 91.1	24/10	150.2 > 119.1	-	-	1.8 ± 0.1	-	Methamphetamine-D5
Benzylpiperazine (BZP)	35/20	177.1 > 91.1	35/15	177.1 > 85.1	-	-	6.5 ± 0.6	-	PCP-D5
MDA	21/11	180.0 > 163.1	21/22	180.0 > 105.1	-	-	2.6 ± 0.4	-	MDA-D5
MDMA	24/13	194.1 > 163.1	24/24	194.1 > 105.1	-	-	2.1 ± 0.1	-	MDMA-D5
MDEA	28/13	208.1 > 163.1	28/27	208.1 > 105.1	-	-	2.1 ± 0.2	-	MDEA-D5
HMA	6/14	182.1 > 165.0	6/24	182.1 > 105.0	6/18	182.1 > 133.0	1.8 ± 0.7	2.4 ±1.4	Amphetamine-D5
HMMA	16/12	196.1 > 165.0	16/26	196.1 > 105.0	16/22	196.1 > 133.0	3.1 ± 0.6	3.8 ±0.6	Methamphetamine-D5
DHMA	6/12	182.1 > 151.0	6/18	182.1 > 123.0	6/24	182.1 > 105.0	2.8 ± 0.5	3.2 ±0.7	Amphetamine-D5
Mephedrone	10/12	178.1 > 160.1	10/22	178.1 > 145.0	10/22	178.1 > 119.0	1.6 ± 0.2	8.5 ±2.1	Mephedrone-D3
p-Methoxyamphetamine (PMA)	20/20	166.0 > 121.0	20/20	166.0 > 149.0	-	-	12.5 ± 1.5	-	MDA-D5
Heroin	51/50	370.2 > 165.1	51/29	370.2 > 268.1	-	-	1.5 ± 0.2	-	Heroin-D9
O-6-monoacetylmorphine (O-6-MAM)	52/39	328.1 > 165.1	52/26	328.1 > 211.1	-	-	1.4 ± 0.3	-	PCP-D5
Codeine	49/25	300.2 > 215.1	49/57	300.2 > 152.1	-	-	1.8 ± 0.1	-	Codeine-D6
Oxycodone	36/29	316.2 > 241.1	36/26	316.2 > 256.1	-	-	1.4 ± 0.3	-	Oxycodone-D6
Noroxycodone	22/36	302.1 > 227.0	22/28	302.1 > 187.0	-	-	5.5 ± 0.8		Oxycodone-D6
Hydrocodone	24/34	300.1 > 199.0	24/46	300.1 > 171.0	-	-	3.7 ± 0.2	-	Hydrocodone-D6
Oxymorphone	40/19	302.1 > 284.1	40/28	302.1 > 227.1	-	-	2.3 ± 0.2	-	Oxycodone-D6
Morphine	53/38	286.1 > 165.1	53/56	286.1 > 152.1	-	-	1.2 ± 0.2	-	Morphine-D6
Normorphine	45/43	272.1 > 165.0	45/49	272.1 > 152.1	-	-	1.3 ± 0.5	-	Morphine-D6
Dihydromorphine	28/42	288.2 > 185.0	28/32	288.2 > 213.0	28/42	288.2 > 231.0	2.9 ± 0.5	129.6 ±68.4	Morphine-D6
Dihydrocodeine	53/33	302.1 > 199.1	53/60	302.1 > 128.1	-	-	1.9 ± 0.2	-	Codeine-D6
Morphine-3β-D-glucuronide	56/44	462.3 > 286.1	56/80	462.3 > 165.0	56/56	462.3 > 201.1	4.7 ± 1.8	9.88 ±3.0	Morphine-3β-D-glucuronide-D3

Methadone	31/15	310.2 > 265.1	31/28	310.2 > 105.1	-	-	1.6 ± 0.5	-	Methadone-D9
EDDP	50/29	278.2 > 234.1	50/24	278.2 > 249.1	-	-	2.3 ± 0.1	-	EDDP-D3
Tramadol	24/17	264.2 > 58.1	24/11	264.2 > 246.3	-	-	102.1 ± 3.6	-	Methamphetamine-D5
O-desmethyl-tramadol	2/18	250.1 > 58.0	2/20	250.1 > 232.0	2/34	250.1 > 107.0	899.7 ± 14.7	1228.0 ±373.0	Codeine-D6
Temazepam	37/21	301.1 > 255.1	37/14	301.1 > 283.1	-	-	2.2 ± 0.1	-	Temazepam-D5
Diazepam	54/27	285.0 > 154.1	54/31	285.0 > 193.1	-	-	1.2 ± 0.1	-	Diazepam-D5
Nordiazepam	51/29	271.1 > 140.1	51/29	271.1 > 165.0	-	-	2.0 ± 0.1	-	Nordiazepam-D5
Nitrazepam	44/24	282.1 > 236.1	44/37	282.1 > 180.1	-	-	2.5 ± 0.3	-	Nitrazepam-D5
7-aminonitrazepam	48/25	252.1 > 121.1	48/40	252.1 > 94.1	-	-	5.7 ± 0.9	-	Nitrazepam-D5
Oxazepam	38/21	287.1 > 241.1	38/15	287.1 > 269.0	-	-	1.3 ± 0.	-	Oxazepam-D4
Lorazepam	30/20	321.0 > 275.1	30/33	321.0 > 229.1	-	-	3.3 ± 1.4	-	Lorazepam-D4
Zopiclone	22/18	389.1 > 245.0	22/42	389.1 > 217.0	-	-			Zopiclone-D4
Zolpidem	8/36	308.2 > 235.2	8/36	308.2 > 263.0	-	-	1.7 ± 0.5		Cocaine-D3
Amitriptyline	37/26	278.2 > 91.1	37/18	278.2 > 233.2	-	-	1.8 ± 0.2	-	EDDP-D3
Fluoxetine	25/8	310.3 > 148.1	-	-	-	-	-	-	MDMA-D5
Norfluoxetine	17/7	296.2 > 134.1	-	-	-	-	-	-	MDMA-D5
Venlafaxine	27/12	278.2 > 58.1	27/12	278.2 > 260.1	27/32	278.2 > 121.0	2.7 ± 0.2	4.5 ±0.7	Methamphetamine-D5
Desmethylvenlafaxine	25/24	264.0 > 58.1	25/24	264.0 > 107.1	25/20	264.0 > 246.3	12.7 ± 1.8	66.4 ±5.9	Methamphetamine-D5
Ketamine	31/27	238.1 > 125.0	31/15	238.1 > 220.1	-	-	3.3 ± 0.5	-	Ketamine-D4
Norketamine	23/27	224.0 > 125.0	23/12	224.0 > 207.1	-	-	1.1 ± 0.1	-	Norketamine-D4
Sildenafil	60/28	475.3 > 100.2	68/50	475.3 > 283.2	68/36	475.3 > 311.2	28.6 ± 8.6	17.3 ±4.0	PCP-D5
Vardenafil	74/68	489.3 > 151.0	74/48	489.3 > 321.1	-	-	7.2 ± 3.0	-	Methadone-D9
Ephedrine	23/12	166.1 > 148.1	23/21	166.1 > 133.0	-	-	7.4 ± 0.8	-	1S, 2R-(+)-ephedrine-D3
Pseudoephedrine	23/12	166.1 > 148.1	23/21	166.1 > 133.0	-	-	6.9 ± 0.6	-	1S, 2R-(+)-ephedrine-D3
Norephedrine	23/10	152.1 > 134.1	23/16	152.1 > 117.1	-	-	3.1 ± 0.4	-	1S, 2R-(+)-ephedrine-D3
Caffeine	38/15	195.1 > 138.0	38/23	195.1 > 110.0	-	-	2.5 ± 0.3	-	Cotinine-D3
1,7-dimethylxanthine	54/21	181.0 > 124.1	-	-	-	-	-	-	Cotinine-D3
Nicotine	37/20	163.1 > 130.0	37/24	163.1 > 117.0	-	-	1.4 ± 0.1	-	Cotinine-D3
Cotinine	34/21	177.1 > 80.0	34/22	177.1 > 98.1	-	-	2.8 ± 0.2	-	Cotinine-D3
Creatinine	31/11	114.0 > 86.1	31/16	114.0 > 72.1	-	-	21.9 ± 4.2	-	Cotinine-D3

[a]CV, cone voltage (V); CE, collision energy (eV)

Table 3 MRM transitions selected for internal standards used in the method

Internal Standards	CV/CE[a]	MRM1 (quantification)
Cocaine-D3	40/20	307.2 > 185.1
Benzoylecgonine-D8	38/19	298.2 > 171.1
Cocaethylene-D3	42/20	321.2 > 199.1
Ecgonine Methyl ester-D3	44/22	203.2 > 185.2
Amphetamine-D5	22/16	141.0 > 92.9
Methamphetamine-D5	28/12	155.1 > 121.0
PCP-D5	18/14	249.2 > 164.1
Mephedrone-D3	30/22	181.1 > 163.1
MDA-D5	21/11	185.1 > 168.1
MDMA-D5	26/13	199.1 > 165.1
MDEA-D5	28/13	213.1 > 163.0
Cotinine-D3	44/24	180.1 > 80.0
EDDP-D3	50/29	281.2 > 234.1
Heroin-D9	51/50	379.2 > 165.8
Codeine-D6	52/28	306.2 > 218.1
Oxycodone-D6	36/29	322.2 > 247.1
Hydrocodone-D6	64/32	306.2 > 202.0
Morphine-D6	53/38	292.2 > 153.1
Morphine-3β-D-glucuronide-D3	52/36	465.2 > 289.1
Methadone-D9	31/15	319.3 > 268.2
Temazepam-D5	37/21	306.7 > 260.1
Diazepam-D5	54/27	290.1 > 154.1
Nordiazepam-D5	48/36	276.1 > 140.1
Nitrazepam-D5	52/42	287.1 > 185.0
Oxazepam-D4	38/21	292.0 > 246.0
Lorazepam-D4	25/29	325.0 > 279.2
Zopiclone-D4	24/16	393.1 > 245.0
Ketamine-D4	31/27	242.1 > 129.1
Norketamine-D4	32/28	228.1 > 128.9
1S,2R-(+)-Ephedrine-D3	23/18	169.2 > 151.0

[a]CV, cone voltage (V); CE, collision energy (eV)

Table 4 SPE recovery for the studied analytes

Analyte	**SPE relative recovery % (n=3)**		
	25 ng/L*	250 ng/L*	2500 ng/L*
Cocaine	100.0 ± 1.9	91.0 ± 0.7	85.0 ± 1.7
Benzoylecgonine	76.0 ± 1.4	79.0 ± 1.8	98.0 ± 3.9
Cocaethylene	102.0 ± 2.6	92.0 ± 0.2	94.0 ± 1.4
R-(-)-Amphetamine	101.0 ± 6.6	76.0 ± 1.6	82.0 ± 4.7
S-(+)-Amphetamine	81.0 ± 10.6	99.0 ± 2.0	82.0 ± 4.2
R-(-)-Methamphetamine	91.0 ± 4.4	113.0 ± 0.7	82.0 ± 5.0
S-(+)-Methamphetamine	84.0 ± 1.9	86.0 ± 1.2	84.0 ± 7.1
E1-Mephedrone	109.0 ± 3.2	99.0 ± 4.8	80.0 ± 7.0
E2-Mephedrone	99.0 ± 8.5	99.0 ± 4.3	87.0 ± 11.5
R-(-)-MDA	93.0 ± 6.2	94.0 ± 4.2	81.0 ± 1.0
S-(+)-MDA	110.0 ± 8.5	99.0 ± 1.5	91.0 ± 1.5
R-(-)-MDMA	91.0 ± 3.7	81.0 ± 7.8	89.0 ± 4.3
S-(+)-MDMA	93.0 ± 1.7	100.0 ± 0.7	84.0 ± 1.9
E1-MDEA	102.0 ± 2.0	95.0 ± 8.6	91.0 ± 5.9
E2-MDEA	99.0 ± 1.8	92.0 ± 1.9	93.0 ± 13.4
Heroin	86.0 ± 9.4	80.0 ± 5.6	75.0 ± 2.4
O-6-monoacetylmorphine	108.0 ± 2.3	120.0 ± 1.4	114.0 ± 1.0
Morphine	98.0 ± 15.2	92.0 ± 1.3	112.0 ± 3.2
Morphine-3β-D-glucuronide	99.0 ± 0.5	121.0 ± 2.5	109.0 ± 5.3
Ketamine	127.0 ± 2.5	100.0 ± 5.7	85.0 ± 6.2
Benzylpiperazine	112.0 ± 5.3	96.0 ± 13.1	100.0 ± 4.9
Temazepam	117.0 ± 4.1	117.0 ± 3.0	99.0 ± 4.7
Diazepam	93.0 ± 3.9	115.0 ± 0.3	95.0 ± 4.9
Nordiazepam	108.0 ± 9.0	108.0 ± 11.2	96.0 ± 4.7
Nitrazepam	89.0 ± 3.1	91.0 ± 2.7	89.0 ± 1.5
Oxazepam	92.0 ± 2.7	117.0 ± 1.4	92.0 ± 1.6
7-amino-nitrazepam	83.0 ± 6.4	85.0 ± 0.4	80.0 ± 4.1
Lorazepam	98.0 ± 14.4	108.0 ± 3.4	86.0 ± 3.0
Anhydroecgonine methyl ester	80.0 ± 5.3	102.0 ± 0.1	86.0 ± 0.4
E1-HMA	97.0 ± 8.7	114.0 ± 0.3	106.0 ± 16.4
E2-HMA	106.0 ± 4.6	107.0 ± 2.9	120.0 ± 11.5
E1-HMMA	84.0 ± 8.8	85.0 ± 9.4	100.0 ± 3.3
E2-HMMA	108.0 ± 7.5	105.0 ± 2.4	118.0 ± 1.7
DHMA	108 ± 11.9	112 ± 2.4	111 ± 2.8
Caffeine	80.0 ± 2.5	84.0 ± 2.7	80.0 ± 1.5
1,7-dimethylxanthine	104.0 ± 0.5	100.0 ± 1.3	106.0 ± 2.4
Nicotine	97.0 ± 2.5	81.0 ± 6.2	120.0 ± 9.5
Cotinine	105.0 ± 2.8	93.0 ± 6.1	89.0 ± 3.5
Creatinine	80.0 ± 4.6	94.0 ± 9.5	109.0 ± 13.3
Codeine	95.0 ± 6.7	108.0 ± 3.1	107.0 ± 2.1
Oxycodone	84.0 ± 2.1	91.0 ± 3.8	99.0 ± 3.3
Noroxycodone	93.0 ± 11.3	80.0 ± 2.8	90.0 ± 3.2
Hydrocodone	84.0 ± 3.1	104.0 ± 8.9	101.0 ± 10.6
Oxymorphone	94.0 ± 5.7	87.0 ± 7.7	89.0 ± 1.2
Dihydrocodeine	98.0 ± 7.3	104.0 ± 2.9	89.0 ± 3.8
Methadone	95.0 ± 0.9	116.0 ± 0.5	89.0 ± 1.4
EDDP	90.0 ± 6.5	97.0 ± 3.0	90.0 ± 1.1
E1-Venlafaxine	83.0 ± 0.6	105.0 ± 6.3	91.0 ± 0.4
E2-Venlafaxine	91.0 ± 5.8	104.0 ± 5.4	90.0 ± 0.7
Vardenafil	120.0 ± 0.5	115.0 ± 11.0	100.0 ± 8.8
E1-Norephedrine	112.0 ± 2.8	117.0 ± 1.1	108.0 ± 1.5
E2-Norephedrine	115.0 ± 5.9	95.0 ± 2.1	83.0 ± 1.4
E1-PMA	110.0 ± 8.5	94.0 ± 2.4	80.0 ± 0.7
E2-PMA	113.0 ± 3.5	118.0 ± 5.9	91.0 ± 0.4
Normorphine	80.0 ± 8.4	80.0 ± 11.9	111.0 ± 4.0

Dihydromorphine	106.0 ± 0.5	80.0 ± 2.0	80.0 ± 4.5
D1-Tramadol	109.0 ± 6.0	111.0 ± 7.2	96.0 ± 10.0
D2-Tramadol	90.0 ± 7.8	81.0 ± 2.7	80.0 ± 1.1
O-Demethyltramadol	80.0 ± 6.4	118.0 ± 4.4	80.0 ± 3.3
Zolpidem	101.0 ± 0.8	96.0 ± 14.0	115.0 ± 1.7
Amitriptyline	81.0 ± 0.2	82.0 ± 2.9	92.0 ± 2.7
Norketamine	89.0 ± 8.2	116.0 ± 2.6	102.0 ± 2.2
Sildenafil	115.0 ± 0.7	105.0 ± 8.5	96.0 ± 9.0
(+)-Ephedrine	81.0 ± 9.0	82.0 ± 2.6	91.0 ± 2.1
(-)-Ephedrine and (-)-Ψephedrine	112.0 ± 0.6	87.0 ± 2.5	113.0 ± 9.6
(+)-Ψephedrine	104.0 ± 10.6	83.0 ± 0.3	81.0 ± 1.0
Desmethylvenlafaxine-E1	91.0 ± 9.8	113.0 ± 14.2	98.0 ± 6.5
Desmethylvenlafaxine-E2	82.0 ± 1.1	92.0 ± 4.1	99.0 ± 10.7
E1-Zopiclone	80.0 ± 2.0	82.0 ± 0.7	81.0 ± 3.7
E2-Zopiclone	80.0 ± 1.2	80.0 ± 6.7	83.0 ± 4.6
S-(+)-Fluoxetine	100.0 ± 5.5	81.0 ± 3.8	100.0 ± 0.7
R-(-)-Fluoxetine	97.0 ± 16.6	91.0 ± 5.5	101.0 ± 7.1
E1-Norfluoxetine	87.0 ± 1.7	80.0 ± 4.6	87.0 ± 5.3
E2-Norfluoxetine	80.0 ± 0.4	81.0 ± 1.7	84.0 ± 2.6

*- the following concentrations were used: 50, 500 and 5000 ng L^{-1} in the case of compounds that were not enantioseparated

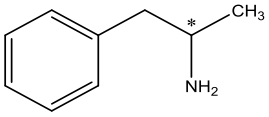
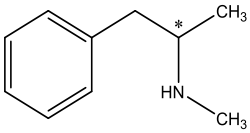
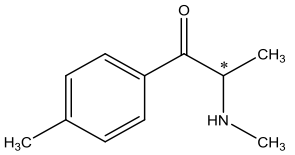
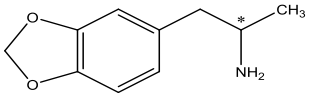
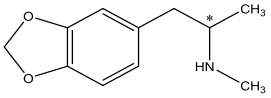
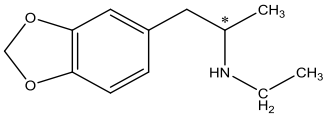
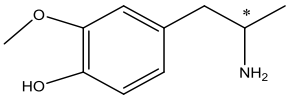
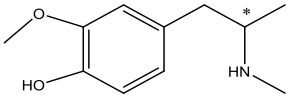
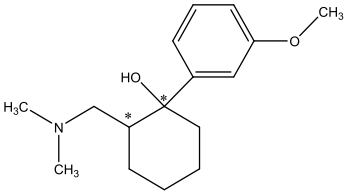
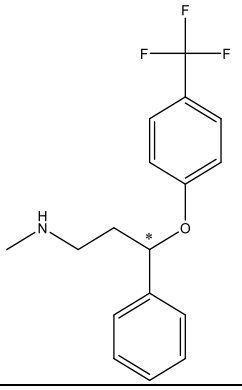
Table 5 Validation parameters - retention time, relative retention time, linearity range, correlation coefficient obtained from calibration curve and instrumental and method limits of detection and instrumental and method limits of quantification

Compound	R_t (min)	Rel. R_t	Sample diluent				WWTP influent	
			Linearity range (µg/L)	R^2	$IDL_{S/N}$ (µg/L)	$IQL_{S/N}$ (µg/L)	MDL (µg/L)	MQL (µg/L)
Cocaine	15.7 ±0.4	0.3	0.010-1000	0.9997	0.01	0.05	0.0001	0.0003
Benzoylecgonine	3.1 ±0.0	0.0	0.005-1000	0.9992	0.01	0.02	0.0001	0.0001
Cocaethylene	16.0 ±0.7	0.3	0.100-1000	0.9996	0.10	0.25	0.0005	0.0013
R-(-)-Amphetamine	15.5 ±0.3	0.1	0.125-500	0.9987	0.12	0.50	0.0008	0.0029
S-(+)-Amphetamine	22.6 ±0.4	0.2	0.125-500	0.9988	0.12	0.50	0.0008	0.0029
R-(-)-Methamphetamine	14.5 ±0.4	0.3	0.050-500	0.9989	0.05	0.12	0.0003	0.0006
S-(+)-Methamphetamine	16.5 ±0.4	0.3	0.050-500	0.9994	0.05	0.12	0.0003	0.0007
E1-Mephedrone	16.5 ±0.4	0.3	0.250-500	0.9990	0.25	0.50	0.0013	0.0026
E2-Mephedrone	21.0 ±0.5	0.2	0.250-500	0.9993	0.25	0.50	0.0007	0.0026
R-(-)-MDA	28.1 ±0.5	0.2	0.500-500	0.9991	0.50	2.50	0.0028	0.0140
S-(+)-MDA	47.4 ±0.8	0.4	0.500-500	0.9980	0.50	2.50	0.0025	0.0124
R-(-)-MDMA	21.9 ±0.5	0.2	0.050-500	0.9992	0.05	0.25	0.0003	0.0014
S-(+)-MDMA	32.9 ±0.5	0.1	0.050-500	0.9994	0.05	0.25	0.0003	0.0013
E1-MDEA	19.0 ±0.5	1.8	0.125-500	0.9994	0.12	0.25	0.0006	0.0013
E2-MDEA	21.0 ±0.5	0.2	0.125-500	0.9995	0.12	0.25	0.0007	0.0013
Heroin	22.5 ±0.4	0.5	1.000-1000	0.9946	1.00	5.00	0.0062	0.0312
O-6-monoacetylmorphine	24.1 ±0.7	1.1	0.250-1000	0.9987	0.25	1.00	0.0011	0.0044
Morphine	17.4 ±0.8	0.5	0.250-1000	0.9955	0.25	0.50	0.0012	0.0025
Morphine-3β-D-glucuronide	3.3 ±0.0	6.5	0.500-1000	0.9983	0.50	5.00	0.0023	0.0228
Ketamine	11.6 ±0.2	0.3	0.100-1000	0.9994	0.10	0.25	0.0005	0.0012
Benzylpiperazine	21.3 ±0.4	1.6	0.500-1000	0.9957	0.50	1.00	0.0024	0.0048
Temazepam	5.2 ±0.2	1.2	0.250-1000	0.9972	0.25	0.50	0.0011	0.0022
Diazepam	6.0 ±0.3	0.6	0.250-1000	0.9974	0.25	0.50	0.0012	0.0024
Nordiazepam	8.9 ±0.2	0.5	0.250-1000	0.9985	0.25	0.50	0.0012	0.0024
Nitrazepam	7.3 ±0.0	1.4	0.250-1000	0.9984	0.25	0.50	0.0014	0.0027
Oxazepam	7.0 ±0.2	4.8	0.500-1000	0.9971	0.50	1.00	0.0025	0.0049
7-amino-nitrazepam	5.3 ±0.1	0.6	0.250-1000	0.9923	0.25	0.50	0.0015	0.0030
Lorazepam	6.8 ±0.1	1.4	1.000-800	0.9900	1.00	5.00	0.0051	0.0256
Anhydroecgonine methyl ester	12.4 ±0.2	0.5	0.500-1000	0.9971	0.50	1.00	0.0028	0.0056
E1-HMA	17.7 ±0.4	0.4	2.500-500	0.9900	2.50	5.00	0.0118	0.0236
E2-HMA	34.3 ±0.5	0.8	2.500-500	0.9903	2.50	5.00	0.0113	0.0225

E1-HMMA	15.9 ±0.4	2.5	0.250-500	0.9982	0.25	0.50	0.0014	0.0028
E2-HMMA	18.6 ±0.5	2.5	0.250-500	0.9974	0.25	0.50	0.0011	0.0022
DHMA	12.5 ±0.2	4.1	1.000-1000	0.9959	1.00	5.00	0.0045	0.0226
Caffeine	6.1 ±0.0	0.8	0.250-1000	0.9981	0.25	0.50	0.0047	0.0259
1,7-Dimethylxanthine	6.4 ±0.1	0.8	1.000-1000	0.9983	1.00	5.00	0.0048	0.0241
Nicotine	12.5 ±0.1	2.6	0.250-1000	0.9964	0.25	0.50	0.0013	0.0025
Cotinine	3.4 ±0.0	0.5	0.010-1000	0.9988	0.01	0.02	0.0001	0.0001
Creatinine	3.0 ±0.0	2.0	1.000-1000	0.9943	1.00	5.00	0.0053	0.0265
Codeine	15.8 ±0.4	0.3	0.500-1000	0.9980	0.50	1.00	0.0024	0.0048
Oxycodone	16.1 ±0.7	0.3	0.250-1000	0.9977	0.25	1.00	0.0014	0.0054
Noroxycodone	20.7 ±0.3	0.6	1.000-1000	0.9991	1.00	5.00	0.0057	0.0285
Hydrocodone	19.2 ±1.1	0.4	1.000-1000	0.9987	1.00	5.00	0.0052	0.0259
Oxymorphone	18.7 ±0.5	0.3	1.000-1000	0.9976	1.00	5.00	0.0056	0.0278
Dihydrocodeine	14.0 ±0.5	0.6	0.500-1.000	0.9985	0.50	1.00	0.0026	0.0051
Methadone	21.3 ±1.3	0.3	0.250-1000	0.9992	0.25	0.50	0.0012	0.0025
EDDP	19.7 ±0.5	0.2	0.025-1000	0.9993	0.02	0.10	0.0001	0.0005
E1-Venlafaxine	12.5 ±0.5	0.6	0.125-500	0.9980	0.12	0.25	0.0007	0.0013
E2-Venlafaxine	15.6 ±0.5	2.9	0.125-500	0.9971	0.12	0.25	0.0007	0.0013
Vardenafil	24.7 ±1.3	2.8	1.000-1000	0.9911	1.00	5.00	0.0045	0.0223
E1-Norephedrine	13.6 ±0.3	0.4	0.125-500	0.9981	0.12	0.25	0.0006	0.0011
E2-Norephedrine	15.1 ±0.4	2.2	0.125-500	0.9983	0.12	0.25	0.0006	0.0012
E1-PMA	21.3 ±0.5	0.5	0.125-500	0.9964	0.12	0.25	0.0007	0.0013
E2-PMA	36. 8 ±0.4	1.4	0.125-500	0.9994	0.12	0.25	0.0006	0.0011
Normorphine	20.0 ±0.6	0.8	1.000-800	0.9905	1.00	5.00	0.0055	0.0276
Dihydromorphine	15.1 ±0.5	0.5	1.000-800	0.9915	1.00	5.00	0.0056	0.0282
D1-Tramadol	12.6 ±0.4	0.6	0.500-500	0.9985	0.50	1.00	0.0024	0.0047
D2-Tramadol	13.7 ±0.5	0.7	0.500-500	0.9989	0.50	1.00	0.0029	0.0059
O-Demethyltramadol	13.5 ±0.4	0.8	0.500-1000	0.9921	0.50	1.00	0.0027	0.0053
Zolpidem	15.1 ±0.6	2.3	0.025-1000	0.9924	0.02	1.00	0.0001	0.0047
Amitriptyline	55.3±3.1	2.9	5.000-1000	0.9950	5.00	10.00	0.0294	0.0588
Norketamine	8.5 ±0.3	0.6	0.500-1000	0.9986	0.50	1.00	0.0024	0.0048
Sildenafil	17.7 ±1.0	3.8	1.000-1000	0.9911	1.00	5.00	0.0047	0.0237
(+)-Ephedrine	12.3 ±0.3	0.6	1.000-500	0.9974	1.00	5.00	0.0059	0.0295
(-)-Ephedrine and (-)-Ψephedrine	13.4 ±0.	0.5	0.500-1000	0.9975	0.50	1.00	0.0024	0.0048
(+)-Ψephedrine	32.94 ±0.8	1.9	1.000-500	0.9903	1.00	5.00	0.0056	0.0280
Desmethylvenlafaxine-E1	15.8 ±0.4	0.7	5.000-500	0.9941	5.000	10.000	0.0249	0.0497
Desmethylvenlafaxine-E2	17.2 ±0.4	0.6	5.000-500	0.9973	5.000	10.000	0.0275	0.0550
E1-Zopiclone	32.7 ±0.3	4.6	10.000-500	0.9903	10.000	50.000	0.0285	0.3125

E2-Zopiclone	59.8 ±0.4	5.2	10.000-500	0.9909	10.000	50.000	0.0326	0.3208
S-(+)-Fluoxetine	43.2 ±1.8	3.1	10.000-500	0.9915	10.000	50.000	0.0533	0.2664
R-(-)-Fluoxetine	57.2 ±2.1	3.3	10.000-500	0.9907	10.000	50.000	0.0517	0.2588
E1-Norfluoxetine	81.3 ±6.0	14.4	10.000-500	0.9916	10.000	50.000	0.0589	0.2945
E2-Norfluoxetine	87.8 ±3.5	12.9	10.000-500	0.9921	10.000	50.000	0.0612	0.3061

Table 6 Validation parameters - enantiomeric fraction (EF) and enantiomeric resolution (Rs) of compounds, which enantiomers were separated under studied conditions.

	Structure	Rs	EF (n=9)		
			10 µg/L	100 µg/L	1000 µg/L
Amphetamine		1.2±0.1	0.47±0.01	0.49±0.02	0.48±0.01
Methamphetamine		1.0±0.0	0.50±0.00	0.49±0.00	0.49±0.00
Mephedrone		1.4±0.1	0.50±0.01	0.50±0.00	0.48±0.01
MDA		1.8±0.2	0.47±0.01	0.48±0.01	0.50±0.00
MDMA		1.2±0.1	0.51±0.00	0.50±0.00	0.51±0.00
MDEA		0.8±0.3	0.50±0.01	0.51±0.01	0.50±0.01
HMA		2.7±0.3	0.54±0.08	0.47±0.00	0.49±0.05
HMMA		0.8±0.1	0.48±0.01	0.43±0.01	0.40±0.00
Tramadol		0.9±0.0	0.46±0.01	0.46±0.02	0.49±0.03
Fluoxetine		0.6±0.2	0.51±0.03	0.50±0.02	0.51±0.04

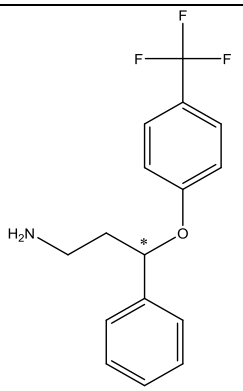
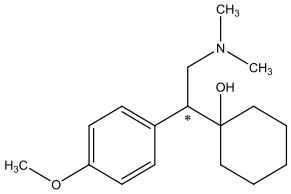
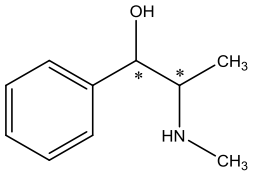
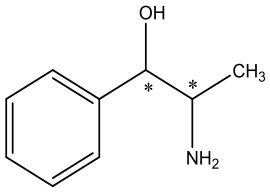
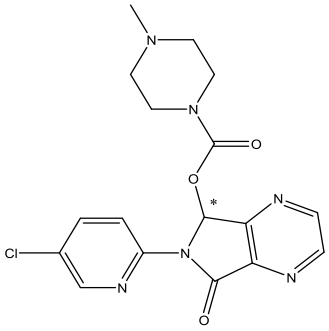
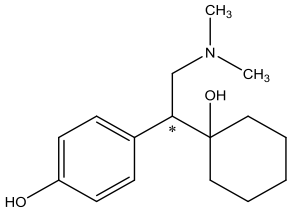
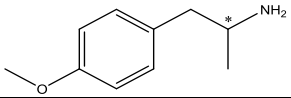
Norfluoxetine		1.9±0.10	0.50±0.08	0.47±0.05	0.50±0.07
Venlafaxine		1.0±0.1	0.50±0.04	0.49±0.01	0.50±0.01
(+)-Ephedrine		0.9±0.1	0.40±0.03	0.50±0.1	0.49±0.16
(+)-Pseudoephedrine		2.2±0.2	0.52±0.01	0.45±0.03	0.42±0.02
Norephedrine		0.9±0.1	0.50±0.04	0.46±0.01	0.47±0.00
Zopiclone		3.1±0.2	0.43±0.02	0.47±0.01	0.48±0.02
Desmethylvenlafaxine		0.9±0.1	0.42±0.04	0.43±0.03	0.40±0.03
PMA		2.7±0.2	0.48±0.02	0.47±0.01	0.41±0.00

Table 7 Validation parameters - method precision

Analytes	Intra-day RSD% (n=4)									Inter-day RSD% (n=3)		
	25 ng/L** D 1*	25 ng/L D 2	25 ng/L D 3	250 ng/L D 1	250 ng/L D 2	250 ng/L D 3	2500 ng/L D 1	2500 ng/L D 2	2500 ng/L D 3	25 ng/L	250 ng/L	2500 ng/L
Cocaine	6.5	2.7	5.2	0.5	4.8	3.4	4.7	1.9	1.5	4.8	2.9	2.7
Benzoylecgonine	2.3	7.5	10.2	5.3	4.6	6.6	14.4	2.5	4.9	6.6	5.5	7.3
Cocaethylene	3.5	5.1	5.4	6.4	3.8	3.8	6.1	2.0	4.3	4.7	4.7	4.2
R-(-)-Amphetamine	3.3	2.5	4.6	5.2	14.7	10.8	6.2	3.9	6.2	3.5	10.2	5.4
S-(+)-Amphetamine	3.1	4.3	12.6	1.4	6.5	4.7	3.8	7.0	7.3	6.7	4.2	6.0
R-(-)-Methamphetamine	8.9	6.7	9.3	3.4	7.0	8.3	4.8	5.2	5.4	8.3	6.2	5.1
S-(+)-Methamphetamine	6.8	3.6	15.4	1.2	5.5	4.0	2.7	2.9	4.2	8.6	3.6	3.3
E1-Mephedrone	9.8	13.7	14.1	3.6	6.8	14.6	3.7	10.0	5.6	12.5	8.3	6.4
E2-Mephedrone	10.7	12.0	4.6	5.2	12.9	8.4	9.2	3.7	2.8	9.1	8.8	5.2
R-(-)-MDA	1.7	6.6	9.7	3.0	3.4	5.7	0.1	7.7	1.1	6.0	4.0	3.0
S-(+)-MDA	4.4	3.8	7.8	2.6	6.7	5.3	7.2	3.7	4.5	5.3	4.9	5.1
R-(-)-MDMA	7.0	1.8	4.0	5.8	4.6	3.9	3.4	1.5	6.5	4.3	4.8	3.8
S-(+)-MDMA	1.0	1.9	6.9	0.6	3.1	2.9	1.2	2.8	0.7	3.3	2.2	1.6
E1-MDEA	6.9	6.2	3.0	5.1	8.5	7.8	4.7	2.2	4.3	5.4	7.1	3.7
E2-MDEA	6.0	6.3	2.8	1.4	9.2	4.9	8.3	1.4	1.7	5.0	5.2	3.8
Heroin	17.3	12.0	1.1	4.4	6.8	6.4	10.5	5.2	12.2	10.1	5.9	9.3
O-6-monoacetylmorphine	3.3	6.9	12.1	4.2	6.6	5.7	5.8	3.8	6.3	7.4	5.5	5.3
Morphine	17.8	0.8	0.8	8.8	4.5	6.7	6.7	7.2	14.2	6.5	6.7	9.4
Morphine-3β-D-glucuronide	18.2	3.7	23.2	27.1	10.4	4.8	18.6	19.4	4.2	15.0	14.1	14.1
Ketamine	8.3	2.2	3.9	2.0	5.2	3.9	1.9	1.6	2.0	4.8	3.7	1.8
Benzylpiperazine	4.4	1.0	9.4	4.3	7.1	5.2	1.9	4.8	2.1	5.0	5.5	2.9
Temazepam	25.9	16.4	5.5	6.7	8.7	8.5	4.1	3.8	2.7	15.9	8.0	3.5
Diazepam	2.1	5.4	5.8	5.0	9.5	8.4	1.8	3.9	2.7	4.4	7.6	2.8
Nordiazepam	3.1	19.8	7.0	4.7	5.5	5.6	15.7	5.1	6.1	9.9	5.3	9.0
Nitrazepam	9.0	1.2	18.7	9.2	4.5	5.2	6.2	5.1	1.9	9.6	6.3	4.4
Oxazepam	13.7	10.0	10.7	3.9	8.3	5.6	3.4	5.3	7.9	11.4	5.9	5.5
7-amino-nitrazepam	0.0	4.5	5.0	3.4	5.8	2.0	0.0	2.1	5.2	3.2	3.7	2.4

Lorazepam	4.4	10.6	6.7	9.9	3.9	2.4	3.9	6.8	3.3	7.2	5.4	4.7
Anhydroecgonine methyl ester	5.3	9.3	3.6	1.4	5.6	5.7	3.0	3.2	1.0	6.1	4.2	2.4
E1-HMA	4.4	5.1	1.6	7.6	1.1	4.4	6.4	6.0	5.9	3.7	4.4	6.1
E2-HMA	5.2	4.8	12.6	3.8	2.0	5.0	7.0	6.5	6.0	7.5	3.6	6.5
E1-HMMA	7.4	7.6	7.5	2.8	3.8	6.0	4.1	2.7	0.3	7.5	4.2	2.4
E2-HMMA	4.7	6.4	3.6	2.1	2.1	6.2	2.9	3.1	3.6	4.9	3.5	3.2
DHMA	8.9	9.1	1.2	6.1	2.5	9.0	3.2	6.9	4.6	6.4	5.9	4.9
Caffeine	2.2	5.6	2.2	5.1	7.3	3.8	9.5	0.9	1.9	3.3	5.4	4.1
1,7-Dimethylxanthine	6.0	4.0	5.7	4.5	0.0	4.7	2.7	3.5	5.8	5.2	3.1	4.0
(-)-Nicotine	3.7	4.0	8.5	6.6	1.8	4.0	1.6	5.5	5.4	5.4	4.1	4.1
Cotinine	3.0	8.1	6.8	4.4	4.0	4.8	3.2	6.1	4.7	6.0	4.4	4.7
Creatinine	18.3	1.0	9.7	2.7	14.9	8.8	0.7	7.6	4.4	9.6	8.8	4.2
Codeine	2.9	4.5	4.9	2.1	5.7	7.8	6.4	2.7	9.0	4.1	5.2	6.0
Oxycodone	10.5	4.2	16.8	2.3	4.4	8.6	3.3	7.0	7.6	10.5	5.1	6.0
Noroxycodone	19.3	9.2	14.1	2.6	9.3	7.4	4.9	8.3	5.4	14.2	6.4	6.2
Hydrocodone	4.7	1.1	1.9	3.8	9.2	7.1	1.4	5.5	7.8	2.6	6.7	4.9
Oxymorphone	5.3	2.1	6.7	3.7	0.6	3.5	4.9	3.5	6.7	4.7	2.6	5.0
Dihydrocodeine	0.3	7.6	4.7	1.0	7.0	1.7	5.6	3.9	3.7	4.2	3.2	4.4
Methadone	7.8	0.0	7.3	2.1	4.4	6.5	5.9	2.8	5.7	5.0	4.4	4.8
EDDP	3.1	5.3	6.2	3.9	9.4	5.3	5.1	1.1	2.9	4.9	6.2	3.0
E1-Venlafaxine	9.1	1.5	5.7	5.5	5.2	7.5	5.3	7.1	5.6	5.4	6.1	6.0
E2-Venlafaxine	0.0	4.8	3.1	4.9	1.4	7.6	1.5	4.0	5.2	2.6	4.6	3.6
Vardenafil	9.4	11.0	10.6	5.6	9.2	13.0	14.6	9.0	5.2	10.3	9.3	9.6
E1-Norephedrine	7.3	3.8	1.3	2.8	3.0	7.3	4.4	3.0	7.4	4.1	4.3	5.0
E2-Norephedrine	5.7	4.6	6.3	3.1	3.9	6.1	2.2	2.1	3.5	5.5	4.3	2.6
E1-PMA	7.7	4.8	8.3	1.4	4.4	3.7	3.8	4.3	5.3	6.9	3.2	4.5
E2-PMA	6.2	8.8	11.6	7.8	4.6	6.6	1.7	3.9	2.9	8.9	6.3	2.8
Normorphine	11.4	2.9	4.6	2.7	12.6	5.5	12.0	3.6	7.8	6.3	6.9	7.8
Dihydromorphine	1.5	13.4	4.1	10.1	14.9	1.4	9.8	2.6	11.3	6.3	8.8	7.9
D1-Tramadol	4.9	7.0	6.6	6.1	5.7	3.9	6.5	1.7	0.5	6.2	5.3	2.9
D2-Tramadol	6.2	9.7	6.1	4.2	3.2	4.0	2.5	3.7	2.5	7.3	3.8	2.9
O-Desmethyltramadol	4.8	8.7	0.0	15.7	16.0	4.6	12.2	16.2	11.5	4.5	12.1	13.3
Zolpidem	18.2	7.0	0.6	5.5	3.4	4.8	0.6	9.1	6.9	8.6	4.5	5.5
Amitriptyline	8.0	7.3	1.1	10.9	7.3	8.7	2.7	6.7	0.1	5.5	9.0	3.1
Norketamine	9.6	7.6	11.2	7.7	8.0	8.3	6.5	5.8	3.6	9.5	8.0	5.3
Sildenafil	20.1	5.9	20.8	1.2	13.3	10.1	5.6	3.5	6.8	15.6	8.2	5.3

(+)-Ephedrine	5.3	16.5	9.8	5.0	4.5	6.6	7.2	2.8	3.3	10.5	5.4	4.4
(-)-Ephedrine and (-)-Ψephedrine	8.3	14.8	5.2	1.8	0.8	5.4	5.7	1.0	3.3	9.4	2.7	3.3
(+)-Ψephedrine	2.8	2.5	6.2	5.8	1.3	9.4	2.9	2.0	1.7	3.8	5.5	2.2
Desmethylvenlafaxine-E1	8.7	7.4	2.3	8.4	3.7	9.5	2.7	5.0	3.7	6.2	7.2	3.8
Desmethylvenlafaxine-E2	6.4	8.7	7.4	3.8	2.8	5.3	2.3	4.9	8.2	7.5	4.0	5.1
E1-Zopiclone	20.0	17.8	19.5	14.5	13.2	19.2	12.6	7.9	5.6	19.1	15.6	8.7
E2-Zopiclone	18.7	18.2	20.4	17.6	14.8	6.9	11.4	5.8	9.8	19.2	13.1	9.0
S-(+)-Fluoxetine	19.3	14.2	1.1	12.9	18.2	14.0	3.5	5.4	2.9	11.5	15.0	4.0
R-(-)-Fluoxetine	19.2	2.7	20.7	6.2	3.8	0.5	0.3	2.7	14.5	14.2	3.5	5.8
E1-Norfluoxetine	17.6	15.1	9.7	6.6	3.8	9.6	2.5	7.9	13.5	14.1	6.7	8.0
E2-Norfluoxetine	1.9	6.8	10.7	20.3	10.5	3.4	0.7	10.7	7.3	6.5	11.4	6.2

*-D indicates day

**- the following concentrations were used: 10, 100 and 1000 ng L^{-1} in the case of compounds that were not enantioseparated

Table 8 Concentration of targeted compounds in wastewater samples during one week monitoring campaign

	Concentration [ng L^{-1}]						
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Cocaine	403 ± 28	449 ± 60	420 ± 13	397 ± 28	452 ± 22	694 ± 23	634 ± 23
Benzoylecgonine	997 ± 150	754 ± 90	788 ± 26	864 ± 112	950 ± 43	1604 ± 129	1537 ± 95
Cocaethylene	4 ± 0	2 ± 0	2 ± 1	2 ± 0	4 ± 0	10 ± 1	9 ± 1
R-(-)-Amphetamine	241 ± 62	169 ± 8	207 ± 39	202 ± 14	204 ± 10	224 ± 17	192 ± 11
S-(+)-Amphetamine	171 ± 12	122 ± 7	154 ± 7	152 ± 14	140 ± 28	170 ± 17	147 ± 3
R-(-)-Methamphetamine	6 ± 1	3 ± 11	6 ± 1	6 ± 2	6 ± 1	5 ± 1	4 ± 1
S-(+)-Methamphetamine	2 ± 2	3 ± 5	6 ± 1	6 ± 4	3 ± 1	3 ± 2	4 ± 1
E1-Mephedrone	42 ± 7	18 ± 6	32 ± 10	18 ± 3	22 ± 7	67 ± 15	53 ± 11
E2-Mephedrone	29 ± 2	14 ± 5	28 ± 3	14 ± 6	18 ± 5	47 ± 6	44 ± 5
R-(-)-MDA	7 ± 7	3 ± 4	10 ± 11	N.D.	N.D.	4 ± 3	7 ± 2
S-(+)-MDA	N.D.	N.D.	2 ± 4	3 ± 4	4 ± 8	13 ± 5	14 ± 7
R-(-)-MDMA	109 ± 7	68 ± 5	45 ± 3	34 ± 2	45 ± 4	133 ± 9	186 ± 10
S-(+)-MDMA	43 ± 4	26 ± 2	23 ± 2	19 ± 2	32 ± 1	84 ± 4	110 ± 6
E1-MDEA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
E2-MDEA	1 ± 0	1 ± 1	N.D.	N.D.	8 ± 16	N.D.	1 ± 1
Heroin	N.D.	26 ± 52	112 ± 223	68 ± 78	50 ± 64	147 ± 24	16 ± 32
O-6-monoacetylmorphine	7 ± 4	2 ± 2	5 ± 4	2 ± 2	4 ± 4	7 ± 5	2 ± 2
Morphine	653 ± 29	643 ± 65	713 ± 40	514 ± 18	640 ± 27	591 ± 63	595 ± 46
Morphine-3β-D-glucuronide	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ketamine	274 ± 17	235 ± 9	284 ± 22	250 ± 14	254 ± 8	287 ± 23	281 ± 14
Benzylpiperazine	9 ± 7	65 ± 6	9 ± 3	9 ± 2	7 ± 1	7 ± 3	8 ± 3
Temazepam	269 ± 78	320 ± 116	408 ± 55	233 ± 125	224 ± 133	256 ± 134	255 ± 119
Diazepam	3 ± 6	2 ± 5	3 ± 3	41 ± 10	22 ± 6	3 ± 6	3 ± 4
Nordiazepam	18 ± 11	9 ± 7	14 ± 11	12 ± 12	12 ± 10	4 ± 8	9 ± 8
Nitrazepam	3 ± 5	29 ± 24	N.D.	4 ± 3	1 ± 2	2 ± 2	4 ± 8
Oxazepam	N.D.	N.D.	184 ± 123	281 ± 198	73 ± 147	98 ± 195	83 ± 96
7-amino-nitrazepam	2 ± 3	28 ± 28	1 ± 1	5 ± 8	5 ± 6	2 ± 3	13 ± 4
Lorazepam	59 ± 59	N.D.	28 ± 35	9 ± 18	33 ± 27	N.D.	5 ± 9
Anhydroecgonine methyl ester	5 ± 1	8 ± 2	8 ± 2	9 ± 2	8 ± 2	12 ± 1	12 ± 1
E1-HMA	43 ± 29	N.D.	N.D.	N.D.	N.D.	13 ± 26	45 ± 35
E2-HMA	46 ± 4	11 ± 22	N.D.	N.D.	N.D.	12 ± 24	32 ± 22
E1-HMMA	27 ± 7	14 ± 2	9 ± 1	7 ± 1	10 ± 2	23 ± 2	35 ± 3
E2-HMMA	21 ± 5	12 ± 3	10 ± 1	9 ± 1	10 ± 2	27 ± 4	33 ± 2
DHMA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Caffeine	184819 ± 14657	135883 ± 11735	173852 ± 8241	171064 ± 8077	171958 ± 5199	169130 ± 5162	151231 ± 5249
1,7-Dimethylxanthine	107717 ± 4786	85882 ± 2418	137196 ± 28326	114869 ± 46279	75413 ± 6759	107717 ± 12820	106272 ± 31432
(-)-Nicotine	6152 ± 4540	3340 ± 653	7810 ± 4460	8562 ± 7806	6375 ± 3844	4872 ± 244	5549 ± 1807
Cotinine	2137 ± 324	1882 ± 202	2116 ± 35	2071 ± 83	2194 ± 67	2266 ± 115	2437 ± 114

Creatinine	679 ± 51	326 ± 87	355 ± 53	379 ± 124	338 ± 79	250 ± 58	330 ± 59
Codeine	2475 ± 56	1914 ± 269	2235 ± 247	1984 ± 195	2079 ± 134	1964 ± 184	1929 ± 257
Oxycodone	11 ± 2	33 ± 30	16 ± 6	14 ± 8	15 ± 4	11 ± 3	18 ± 7
Noroxycodone	21 ± 18	33 ± 23	13 ± 16	15 ± 10	33 ± 5	35 ± 12	25 ± 6
Hydrocodone	N.D.	22 ± 26	14 ± 29	10 ± 20	11 ± 22	38 ± 45	10 ± 20
Oxymorphone	14 ± 12	46 ± 35	20 ± 4	18 ± 2	18 ± 12	12 ± 9	19 ± 3
Dihydrocodeine	449 ± 37	437 ± 83	442 ± 19	380 ± 40	427 ± 36	419 ± 61	406 ± 59
Methadone	54 ± 6	50 ± 11	54 ± 2	53 ± 2	56 ± 4	59 ± 4	51 ± 5
EDDP	126 ± 13	105 ± 10	117 ± 11	106 ± 6	123 ± 16	112 ± 18	122 ± 8
E1-Venlafaxine	94 ± 9	74 ± 12	86 ± 9	92 ± 12	94 ± 9	102 ± 3	105 ± 3
E2-Venlafaxine	122 ± 3	88 ± 8	102 ± 11	97 ± 11	101 ± 12	109 ± 3	92 ± 7
Vardenafil	7 ± 9	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
E1-Norephedrine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
E2-Norephedrine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
E1-PMA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
E2-PMA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Normorphine	148 ± 23	131 ± 79	154 ± 16	181 ± 69	193 ± 52	152 ± 30	145 ± 27
Dihydromorphine	23 ± 5	43 ± 4	25 ± 10	27 ± 3	32 ± 15	15 ± 5	24 ± 5
D1-Tramadol	704 ± 17	720 ± 30	740 ± 48	766 ± 12	692 ± 32	772 ± 40	798 ± 39
D2-Tramadol	640 ± 2	666 ± 21	678 ± 13	621 ± 65	672 ± 26	651 ± 37	595 ± 22
O-Desmethyltramadol	836 ± 76	873 ± 25	950 ± 21	882 ± 20	801 ± 16	849 ± 3	860 ± 9
Zolpidem	1 ± 1	1 ± 2	N.D.	N.D.	N.D.	N.D.	N.D.
Amitriptyline	234 ± 40	126 ± 17	257 ± 29	227 ± 13	232 ± 59	218 ± 6	245 ± 22
Norketamine	47 ± 10	39 ± 9	32 ± 4	57 ± 1	50 ± 14	45 ± 7	37 ± 8
Sildenafil	30 ± 9	3 ± 0	21 ± 7	13 ± 0	12 ± 2	11 ± 2	20 ± 7
(+)-Ephedrine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
(-)-Ephedrine and (-)-Ψephedrine	23 ± 10	21 ± 3	28 ± 4	24 ± 3	27 ± 3	18 ± 7	17 ± 2
(+)-Ψephedrine	201 ± 15	191 ± 10	169 ± 22	163 ± 20	160 ± 19	136 ± 13	153 ± 3
Desmethylvenlafaxine-E1	291 ± 2	296 ± 13	292 ± 14	289 ± 10	315 ± 7	269 ± 15	305 ± 15
Desmethylvenlafaxine-E2	250 ± 6	233 ± 9	235 ± 8	191 ± 4	225 ± 15	211 ± 12	215 ± 26
E1-Zopiclone	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
E2-Zopiclone	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
S-(+)-fluoxetine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
R-(-)-fluoxetine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
E1-Norfluoxetine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
E2-Norfluoxetine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D - not detected

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Table S1 Selected analytes and their properties (MW molecular weight, Exp experimental, Pred predicted, [a] extracted from [38] , [b] predicted using ACD/labs software (http://www.chemspider.com).

Compound	CAS	Formula	MW	pK_a		LogP		LogD[b]		Supplier
				Exp.[a]	Pred.[a]	Exp.[a]	Pred.[b]	pH 5.5	pH 7.4	
Cocaine	50-36-2	$C_{17}H_{21}NO_4$	303.4	8.6 (15°)	8.8	2.3[c]	3.1±0.4	0.1	1.5	LGC (Cerilliant product)
Benzoylecgonine	519-09-5	$C_{16}H_{19}NO_4$	289.3	-	3.1, 9.5	-	2.7±0.4	0.2	0.2	Sigma-Aldrich
Cuscohygrine	454-14-8	$C_{13}H_{24}N_2O$	224.3	-	-	-	0.7±0.3	-3.4	-3.2	TRC
Cocaethylene	529-38-4	$C_{18}H_{23}NO_4$	317.4	-	-	-	2.8	-0.2	1.1	Sigma Aldrich (Cerilliant product)
Anhydroecgonine methyl ester	43021-26-7	$C_{10}H_{15}NO_2$	181.2	-	-	-	1.7±0.3	-0.7	1.0	Sigma Aldrich (Cerilliant product)
(±)-Amphetamine	300-62-9	$C_9H_{13}N$	135.2	10.1 (20°)	10.0	1.8	1.8±0.2	-1.3	-0.6	LGC(Cerilliant product)
(±)-Methamphetamine	4846-07-5	$C_{10}H_{15}N$	149.2	9.9 (25°)	10.2	2.1	1.9±0.2	-1.1	-0.8	LGC (Cerilliant product)
S-(+)-Methamphetamine	537-46-2	$C_{10}H_{15}N$	149.2	9.9 (25°)	10.2	2.1	1.9±0.2	-1.1	-0.8	Cerilliant
BZP (benzylpiperazine)	2759-28-6	$C_{11}H_{16}N_2$	176.3	-	-	-	1.4±0.4	-1.6	-0.4	LGC
TFMPP (1-(3-trifuoromethylphenyl)piperazine)	-	$C_{11}H_{13}F_3N_2$	230.2	-	-	-	2.4±0.5	-0.4	1.2	LGC
(±)-Mephedrone	1189726-22-4	$C_{11}H_{15}NO$	177.7	-	-	-	1.9±0.3	-0.0	1.5	Sigma-Aldrich (Cerilliant product)
PMA (p-Methoxyamphetamine)	3706-26-1	$C_{10}H_{15}NO$	165.0	-	-	-	1.7±0.2	-1.4	-0.8	LGC
(±)-MDA (3,4-methylenedioxyamphetamine)	4764-17-4	$C_{10}H_{13}NO_2$	179.2	9.7 (25°)	10.0	1.6	1.7±0.3	-1.4	-0.8	LGC (Cerilliant product)
(±)-MDMA (3,4-methylenedioxymethamphetamine)	42542-10-9	$C_{11}H_{15}NO_2$	193.2	-	10.1	-1.6,1.9	1.8±0.3	-1.3	-0.9	LGC

(±)-MDEA (3,4-methylenedioxyethylamphetamine)	82801-81-8	$C_{12}H_{17}NO_2$	207.3	-	-	-	2.7±0.3	-0.4	0.3	LGC (Cerilliant product)
D,L-HMA (d,l-4-Hydroxy-3-methoxyamphetamine)	13062-61-8	$C_{10}H_{15}NO_2$	181.2	-	-	-	-	-	-	Kinesis
D,L-HMMA (d,l-4-Hydroxy-3-methoxymethamphetamine)	438625-58-2	$C_{11}H_{17}NO_2$	195.2	-	-	-	1.4	-1.7	-1.2	Kinesis
D,L-3,4-HHMA (2-(3,4-Dihydroxyphenyl)-N-methylpropylamine)	15398-87-5	$C_{10}H_{15}NO_2$	181.2	-	-	-	-	-	-	Kinesis
Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.2	10.4 (40°)	-0.9	-0.1	-0.1±0.4	-0.1	-0.1	Sigma-Aldrich
1,7-dimethylxanthine (Paraxanthine)	611-59-6	$C_7H_8N_4O_2$	180.2	-	-	-	-1.6±0.9	-1.6	-1.6	Sigma-Aldrich
(-)-Nicotine	54-11-5	$C_{10}H_{14}N_2$	162.2	3.1	8.9	1.2	0.7±0.3	-2.1	-0.5	Sigma-Aldrich
(-)-Cotinine	486-56-6	$C_{10}H_{12}N_2O$	176.2	-	-	-	-0.2±0.4	-0.3	-0.2	Sigma Aldrich (Cerilliant product)
Heroin	561-27-3	$C_{21}H_{23}NO_5$	369.4	7.9 (25°)	9.1	1.6	1.5±0.7	-0.8	0.9	Sigma Aldrich (Cerilliant product)
6-acetylmorphine	2784-73-8	$C_{19}H_{21}NO_4$	327.4	-	10.2, 9.1	1.9,1.3	0.4±0.7	-1.7	-0.1	Sigma Aldrich (Cerilliant product)
Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.4	8.2 (25°)	13.8, 9.2	1.2, 1.3	1.2±0.7	-1.4	0.3	Sigma-Aldrich
Norcodeine	467-15-2	$C_{17}H_{19}NO_3$	285.3	-	13.8, 10.1	1.0, 1.0	0.9±0.7	-2.1	-1.1	Sigma Aldrich (Cerilliant product)
Oxycodone	76-42-6	$C_{18}H_{21}NO_4$	315.4	-	13.6, 8.2	1.0, 1.0	1.7±0.6	-0.5	1.2	Sigma Aldrich (Cerilliant product)
Noroxycodone	52446-25--0	$C_{17}H_{19}NO_4$	301.2	-	13.6, 9.5	-	0.2	-2.7	-1.1	Sigma Aldrich (Cerilliant product)

(-)-Oxymorphone	76-41-5	$C_{17}H_{19}NO_4$	301.3	-	10.1, 8.2	-	0.9±0.5	-1.1	0.5	Sigma Aldrich (Cerilliant product)
D-(-)-Morphine	57-27-2	$C_{17}H_{19}NO_3$	285.3	8.2 (25°)	10.3, 9.1	0.9	0.4±0.7	-2.1	-0.4	Sigma Aldrich (Cerilliant product)
Normorphine	466-97-7	$C_{16}H_{17}NO_3$	271.3	-	10.5, 9.8	-	0.1±0.7	-2.9	-1.8	Sigma Aldrich (Cerilliant product)
Dihydromorphine	509-60-4	$C_{17}H_{21}NO_3$	287.4	-	10.3, 9.2	-	0.6±0.4	-2.0	-0.4	Sigma Aldrich (Cerilliant product)
Hydrocodone	125-29-1	$C_{18}H_{21}NO_3$	299.4	-	18.0, 8.6	1.2	1.8±0.5	-0.9	0.7	Sigma Aldrich (Cerilliant product)
Morphine-3β-D-glucuronide	20290-09-9	$C_{23}H_{27}NO_9$	461.5	-	12.2, 10.8	-	-2.0±0.8	-4.5	-4.6	Sigma Aldrich (Cerilliant product)
(±)-Methadone	76-99-3	$C_{21}H_{27}NO$	309.4	8.9 (25°)	18.8, 9.1	3.9	4.2±0.3	1.2	2.6	Sigma Aldrich (Cerilliant product)
(±)-EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)	66729-78-0	$C_{20}H_{23}N$	277.4	-	9.6	-	5.4	3.6	4.9	LGC (Cerilliant product)
(±)-*cis*-Tramadol	36282-47-0	$C_{16}H_{25}NO_2$	263.4	9.4	13.8, 9.2	2.4	2.5±0.3	-0.5	0.5	Sigma-Aldrich
N-Desmethyltramadol	1018989-94-0	$C_{15}H_{23}NO_2$	249.4	-	13.8, 9.9	-	1.7	-1.4	-1.1	LGC
(+)-O-Desmethyltramadol	185453-02-5	$C_{15}H_{23}NO_2$	249.4	-	9.6, 9.0	-	1.7	-1.3	-0.2	LGC
Temazepam	846-50-4	$C_{16}H_{13}ClN_2O_2$	300.7	-	10.7, -1.4	2.2	2.1±0.9	2.1	2.1	Sigma Aldrich (Cerilliant product)
Diazepam	439-14-5	$C_{16}H_{13}ClN_2O$	284.7	3.4	2.9	2.8	2.9±0.9	2.9	2.9	Sigma Aldrich (Cerilliant product)
Nordiazepam	1088-11-5	$C_{15}H_{11}ClN_2O$	270.7	-	12.3, 2.8	2.5[b]	3.1±0.5	3.1	3.1	Sigma Aldrich (Cerilliant product)
Nitrazepam	146-22-5	$C_{15}H_{11}N_3O_3$	281.3	-	11.9, 2.6	2.2	2.2±0.5	2.2	2.2	LGC (Cerilliant product)
7-aminonitrazepam	4928-02-3	$C_{15}H_{13}N_3O$	251.3	-	-	-	1.1±0.8	1.0	1.1	Sigma Aldrich (Cerilliant product)
Oxazepam	604-75-1	$C_{15}H_{11}ClN_2O_2$	286.7	-	10.6, -1.5	-	2.3±0.5	2.3	2.3	Sigma-Aldrich (Cerilliant product)

(±)-Lorazepam	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$	321.2	13	10.6, -2.2	2.4	2.5±0.5	2.5	2.5	Sigma-Aldrich (Cerilliant product)
Amitriptyline	549-18-8	$C_{20}H_{23}N$	277.4	9.4	9.8	4.9	4.9±0.6	1.9	3.1	Sigma-Aldrich
Nortriptyline	894-71-3	$C_{19}H_{21}N$	263.4	-	10.5	-	5.6±0.3	2.6	3.2	Sigma-Aldrich
Fluoxetine	59333-67-4	$C_{17}H_{18}F_3NO$	309.3	-	9.8	4.0	4.1±0.4	1.0	1.6	LGC (Cerilliant product)
R-(-)-fluoxetine	114247-09-5	$C_{17}H_{18}F_3NO$	309.3	-	9.8	4.0	4.1±0.4	1.0	1.6	Sigma-Aldrich
Norfluoxetine	107674-50-0	$C_{16}H_{16}F_3NO$	295.3	-	9.8	-	4.4±0.4	1.4	2.7	LGC (Cerilliant product)
(±)-Venlafaxine	99300-78-4	$C_{17}H_{27}NO_2$	277.4	-	14.4, 8.9	-	2.9±0.3	-0.1	1.2	Sigma-Aldrich
O-Desvenlafaxine	300827-87-6	$C_{16}H_{25}NO_2$	263.0	-	10.1, 8.9	-	2.3±0.3	-0.7	0.5	Sigma-Aldrich
Zolpidem	99294-93-6	$C_{19}H_{21}N_3O$	307.4	6.2	5.6	1.2	3.1±0.6	1.9	3.0	Sigma Aldrich (Cerilliant product)
(±)-Zopiclone	43200-80-2	$C_{17}H_{17}ClN_6O_6$	388.8	-	13.0, 6.9	0.8	-0.3±1.3	-1.5	-0.4	LGC
(±)-Ketamine	1867-66-9	$C_{13}H_{16}ClNO$	237.7	-	18.8, 7.4	2.9	2.2±0.6	1.2	2.1	Sigma-Aldrich
(±)-Norketamine	79499-59-5	$C_{12}H_{14}ClNO$	223.7	-	18.7, 7.5	-	1.9±0.5	1.1	1.9	Sigma Aldrich (Cerilliant product)
Sildenafil	139755-83-2	$C_{22}H_{30}N_6O_4S$	474.6	-	7.3, 6.0	1.9	2.3±1.4	1.6	2.2	Sigma Aldrich (Cerilliant product)
Vardenafil	224789-1515-5	$C_{23}H_{32}N_6O_4S$	488.6	-	8.0, 6.2	1.4	2.6±1.2	1.0	2.5	Sigma Aldrich (Cerilliant product)
(±)-Pentobarbital	76-74-4	$C_{11}H_{18}N_2O_3$	226.3	8.1 (25°)	8.5	2.1	2.0±0.2	2.0	1.9	Sigma-Aldrich (Cerilliant product)
Secobarbital	29071-21-4	$C_{12}H_{18}N_2O_3$	238.3	7.8	8.5	1.9	2.2±0.2	2.2	2.0	Sigma-Aldrich (Cerilliant product)
Ephedrine	50-98-6	$C_{10}H_{15}NO$	165.2	10.3 (0°)	13.9, 9.5	1.1	1.0±0.3	-2.0	-0.9	Sigma-Aldrich
(*1R,2R*)-(-)-Pseudoephedrine	321-97-1	$C_{10}H_{15}NO$	165.2	10.3 (0°)	13.9, 9.5	1.1	1.0±0.3	-2.0	-0.9	Sigma-Aldrich
(±)-Norephedrine	154-41-6	$C_9H_{13}NO$	151.2	9.4 (20°)	13.9, 9.4	0.7	0.8±0.3	-2.2	-1.1	Sigma-Aldrich

Table S2 Studied mobile phase compositions with CHIRALPAK® CBH HPLC

% MP modifiers	Conc. NH_4OAc (mM)	pH
10% IPA	1.0	5.0
10% MeOH	1.0	6.6
10% ACN	1.0	6.4
5% IPA	1.0	6.2
5% MeOH	1.0	6.7
10% MeOH	5.0	6.8
10% MeOH	10.0	6.9
10% MeOH	1.0	6. 7
10% MeOH	2.5	6.6
10% MeOH	1.0	6.2
15% MeOH	1.0	6.4

Table S3 Studied mobile phase compositions with CHIROBIOTIC V

% H_2O	%FA	Conc. NH_4OAc (mM)	pH
1	0.005	4	6.8
5	0.005	4	6.8
20	0.005	4	6.5
80	0.005	4	5.2
25 - 0	0.005	4	
0	0	0	
1	0.005	1	5.9
1	0.005	10	7.4
1	0.001	1	7.3

Table S4 Studied mobile phase compositions with CHIROBIOTIC T (mobile phases with pH<3 were not tested due to the extreme pH not suitable for the studied chiral column)

% H_2O	%FA	Conc. NH_4OAc (mM)	pH
1	0.005	4	6.8
0	0	0	
5	0.005	4	6.8
20	0.005	4	6.5
80	0.005	4	5.2
80	0	20	6.7
0 - 100	0.005	4	
1	0.005	1	5.9
1	0.005	10	7.4
1	0.001	1	7.3
1	0.001	10	7.9
1	0.001	4	7.6
1	0.01	1	5.2
1	0.01	10	6.9
1	0.01	4	6.4
1	1	1	2. 9

1	1	10	3.9
1	1	4	3.5
5	0.005	1	5.6
5	0.005	10	7.0

Table S5 Validation parameters -instrumental precision

	Intra-day RSD% (n=4)									Inter-day RSD% (n=3)		
	5 µg/L** D 1*	5 µg/L D 2	5 µg/L D 3	50 µg/L D 1	50 µg/L D 2	50 µg/L D 3	500 µg/L D 1	500 µg/L D 2	500 µg/L D 3	5 µg/L	50 µg/L	500 µg/L
Cocaine	1.2	2.3	5.3	2.2	4.0	0.5	3.2	3.7	0.5	2.9	2.3	2.5
Benzoylecgonine	3.1	4.1	2.6	6.2	2.9	2.7	1.1	1.8	1.7	3.3	3.9	1.5
Cocaethylene	7.9	3.5	4.8	0.2	0.4	0.6	0.2	3.8	2.4	5.4	0.4	2.1
R-(-)-Amphetamine	4.8	5.8	3.0	2.3	3.1	0.1	3.9	4.7	3.1	4.5	1.9	3.9
S-(+)-Amphetamine	3.7	5.3	6.5	4.6	3.3	4.3	3.2	4.1	3.4	5.2	4.1	3.6
R-(-)-Methamphetamine	6.0	5.8	6.3	3.9	5.5	2.3	3.0	5.1	2.8	6.0	3.9	3.7
S-(+)-Methamphetamine	2.4	2.3	7.7	2.7	0.7	2.1	1.1	4.8	3.4	4.1	1.8	3.1
E1-Mephedrone	9.3	6.7	5.5	1.9	5.7	5.4	2.9	5.5	4.4	7.1	4.3	4.3
E2-Mephedrone	3.5	6.7	1.1	3.6	2.5	2.7	9.3	4.3	2.2	3.8	3.0	5.2
R-(-)-MDA	6.9	1.3	2.7	0.4	5.6	0.1	1.5	0.3	1.6	3.6	2.1	1.1
S-(+)-MDA	5.7	3.2	6.4	8.0	8.9	3.1	0.3	1.1	6.1	5.1	6.7	2.5
R-(-)-MDMA	2.5	5.5	2.0	1.8	6.4	3.9	4.8	3.7	6.1	3.3	4.0	4.9
S-(+)-MDMA	3.5	1.1	4.3	0.5	1.8	1.3	2.5	1.5	2.7	3.0	1.2	2.3
E1-MDEA	8.6	5.3	5.9	2.2	3.8	1.1	6.1	4.3	0.1	6.6	2.4	3.5
E2-MDEA	3.6	2.3	10.3	5.3	1.1	0.4	5.6	1.9	0.7	5.4	2.3	2.7
Heroin	6.7	17.8	0.0	2.4	11.3	29.2	11.5	13.9	13.4	8.2	14.3	13.0
O-6-monoacetylmorphine	9.0	9.2	12.4	0.0	1.8	11.0	1.2	10.7	1.4	10.2	4.3	4.4
Morphine	12.1	4.4	10.6	13.5	5.5	7.9	11.0	1.2	4.4	9.1	8.9	5.5
Morphine-3β-D-glucuronide	14.8	19.9	2.5	4.6	16.8	20.0	8.3	6.6	13.3	12.4	13.8	9.4
Ketamine	4.0	5.4	7.1	2.0	1.4	1.9	1.3	1.8	1.6	5.5	1.8	1.5
Benzylpiperazine	6.8	2.4	2.2	6.6	2.5	10.8	1.1	1.8	3.1	3.8	6.6	2.0
Temazepam	5.9	7.2	7.9	0.7	2.2	6.4	4.4	4.1	0.3	7.0	3.1	2.9
Diazepam	4.9	4.8	6.3	1.2	2.8	4.4	2.3	1.7	1.3	5.3	2.8	1.8
Nordiazepam	7.0	8.4	7.3	5.1	7.5	3.6	2.2	1.6	7.4	7.6	5.4	3.7
Nitrazepam	7.1	4.2	0.8	7.3	7.8	2.9	8.5	5.6	4.7	4.0	6.0	6.3
Oxazepam	7.5	7.7	7.3	2.7	5.5	0.0	1.7	1.6	0.6	7.5	2.7	1.3
7-amino-nitrazepam	3.4	3.6	6.5	4.7	3.3	1.8	4.7	5.0	0.8	4.5	3.2	3.5
Lorazepam	1.9	8.4	21.2	7.5	6.9	1.6	4.8	6.6	7.2	10.5	5.3	6.2
Anhydroecgonine methyl ester	6.8	4.2	2.2	4.4	2.9	4.0	0.7	6.5	2.0	4.4	3.8	3.1
E1-HMA	11.3	5.6	6.3	5.3	6.7	9.1	7.4	4.9	2.1	7.7	7.1	4.8

E2-HMA	6.1	1.7	1.1	3.1	0.4	2.5	8.9	6.3	9.0	3.0	2.0	8.1
E1-HMMA	5.3	8.3	4.1	0.8	6.5	6.6	8.2	4.2	1.7	5.9	4.6	4.7
E2-HMMA	6.6	5.7	9.4	2.4	3.3	7.4	3.8	4.0	4.6	7.2	4.4	4.1
DHMA	8.1	4.5	9.2	3.2	12.3	5.4	3.5	2.6	1.7	7.3	7.0	2.6
Caffeine	1.9	0.9	4.4	3.0	12.9	4.4	4.5	3.6	1.3	2.4	6.7	3.1
1,7-dimethylxanthine	1.5	8.0	7.7	2.8	15.3	6.6	0.2	0.3	0.9	5.7	8.2	0.5
Nicotine	1.6	9.8	8.6	2.7	13.4	2.7	1.7	1.6	1.8	6.7	6.3	1.7
Cotinine	1.1	2.6	6.5	7.0	11.8	2.4	0.1	1.9	4.1	3.4	7.1	2.0
Creatinine	5.2	6.0	7.4	2.3	0.1	1.2	1.1	0.8	1.6	6.2	1.2	1.2
Codeine	11.7	7.3	2.3	7.6	1.7	6.9	6.0	2.6	4.8	7.1	5.4	4.5
Oxycodone	8.3	7.3	4.0	2.5	10.5	6.6	5.6	8.8	5.3	6.5	6.5	6.6
Noroxycodone	1.91	3.3	4.0	6.0	2.3	3.8	1.4	5.8	2.0	3.1	4.0	3.1
Hydrocodone	3.8	5.2	4.7	1.3	7.8	4.1	2.0	0.6	1.3	4.6	4.4	1.3
Oxymorphone	7.3	6.8	5.5	0.4	4.5	2.8	2.1	5.9	2.1	6.5	2.6	3.4
Dihydrocodeine	6.8	8.2	7.4	6.6	8.7	3.4	0.5	2.0	0.6	7.4	6.2	1.0
Methadone	0.6	2.1	1.6	2.5	1.0	3.1	1.3	0.9	0.2	1.4	2.2	0.8
EDDP	5.8	4.8	5.1	1.7	2.7	2.2	0.5	1.3	1.5	5.2	2.2	1.1
E1-Venlafaxine	7.1	3.1	7.4	3.4	2.0	0.8	0.4	1.4	0.9	5.9	2.1	0.9
E2-Venlafaxine	6.1	2.4	0.0	1.9	2.9	3.6	0.5	1.4	0.9	2.8	2.8	0.9
Vardenafil	25.0	7.3	21.8	5.1	1.1	5.1	0.1	0.8	3.1	18.0	3.8	1.3
E1-Norephedrine	5.9	7.1	3.1	2.7	6.9	5.7	2.2	1.3	1.1	5.4	5.1	1.5
E2-Norephedrine	5.4	3.1	4.4	2.4	4.7	3.6	3.4	5.2	2.1	4.3	3.6	3.6
E1-PMA	2.7	8.4	5.6	1.2	8.6	6.1	2.6	0.3	0.4	5.6	5.3	1.1
E2-PMA	4.6	5.4	4.5	4.6	3.9	2.6	3.1	1.6	1.1	4.9	3.7	1.9
Normorphine	4.6	13.3	0.0	20.0	0.5	8.2	3.8	0.1	15.8	6.0	9.5	6.6
Dihydromorphine	18.1	3.0	5.3	15.7	20.0	4.8	8.9	4.9	11.0	8.8	13.5	8.3
D1-Tramadol	11.2	7.1	5.6	3.4	4.1	3.6	5.1	0.9	2.5	8.0	3.7	2.8
D2-Tramadol	2.4	6.8	10.7	13.8	12.4	7.1	2.2	10.0	1.1	6.7	11.1	4.4
O-desmethyltramadol	12.9	10.9	7.2	6.2	3.0	3.5	8.3	5.2	3.2	10.3	4.2	5.6
Zolpidem	15.7	16.3	1.6	1.2	0.1	1.1	3.3	3.4	0.2	11.2	0.8	2.3
Amitriptyline	0.0	10.1	8.3	3.1	6.0	11.3	3.4	3.4	3.4	6.1	6.8	3.4
Norketamine	2.7	6.9	6.0	3.4	1.1	3.5	2.6	2.4	1.4	5.2	2.7	2.1
Sildenafil	5.4	15.7	10.9	4.8	15.3	5.7	7.6	0.9	1.5	10.7	8.6	3.3
(+)-Ephedrine	6.9	3.5	6.5	4.3	5.7	3.2	6.3	1.0	3.4	5.6	4.4	3.6
(-)-Ephedrine and (-)-Ψephedrine	2.6	2.7	4.1	3.6	3.1	2.3	6.4	0.7	4.4	3.1	3.0	3.8
(+)-Ψephedrine	10.6	6.2	5.6	5.9	0.5	2.4	3.4	9.1	3.2	7.4	2.9	5.3
Desmethylvenlafaxine-E1	18.2	7.4	5.2	5.3	7.2	0.7	1.9	8.1	2.0	10.3	4.4	4.0
Desmethylvenlafaxine-E2	5.4	4.5	4.0	5.7	12.5	6.4	2.8	2.0	3.9	4.7	8.2	2.9

E1-Zopiclone	18.2	19.7	15.8	14.2	16.2	12.2	11.4	10.7	8.7	17.9	14.2	10.3
E2-Zopiclone	17.9	19.3	16.7	12.6	18.6	18.0	14.6	13.8	8.5	17.9	16.4	12.3
S-(+)-Fluoxetine	18.3	6.9	4.9	6.0	19.0	9.3	13.6	14.4	2.4	10.0	11.4	10.2
R-(-)-Fluoxetine	16.5	15.9	10.2	13.0	1.3	16.9	1.7	9.3	2.4	14.2	10.4	4.5
E1-Norfluoxetine	11.5	4.4	18.4	5.9	8.2	0.8	5.1	3.1	3.4	11.4	5.0	3.9
E2-Norfluoxetine	10.2	14.3	16.6	15.5	9.8	13.8	17.6	8.0	0.5	13.7	13.1	8.7

*-D indicates day

**- the following concentrations were used: 10, 100 and 1000 ng/L in the case of compounds that were not enantioseparated

Table S6 Validation parameters –ion suppression

	Signal suppression (%) (n=4)
Cocaine	-69.0 ± 25.8
Benzoylecgonine	-6.1 ± 1.5
Cocaethylene	-27.5 ± 5.2
R-(-)-Amphetamine	37.9 ± 9.7
S-(+)-Amphetamine	53.4 ± 9.4
R-(-)-Methamphetamine	-28.5 ± 12.5
S-(+)-Methamphetamine	-6.4 ± 9.2
E1-Mephedrone	-22.3 ± 11.8
E2-Mephedrone	-40.2 ± 14.0
R-(-)-MDA	-15.3 ± 1.4
S-(+)-MDA	-12.5 ± 1.8
R-(-)-MDMA	-43.9 ± 7.4
S-(+)-MDMA	-57.5 ± 8.2
E1-MDEA	-33.9 ± 2.0
E2-MDEA	-58.1 ± 6.6
Heroin	-5.1 ± 10.3
O-6-monoacetylmorphine	-85.2 ± 18.8
Morphine	-58.1 ± 18.5
Morphine-3β-D-glucuronide	99.7 ± 0.6
Ketamine	12.5 ± 11.2
Benzylpiperazine	-50.1 ± 5.3
Temazepam	21.5 ± 4.3
Diazepam	-37.5 ± 7.7
Nordiazepam	-34.9 ± 1.5
Nitrazepam	45.7 ± 2.6
Oxazepam	47.7 ± 4.2
7-amino-nitrazepam	70.9 ± 6.7
Lorazepam	49.3 ± 10.5
Anhydroecgonine methyl ester	-90.4 ± 2.9
E1-HMA	-50.4 ± 6.2
E2-HMA	-68.7 ± 13.9
E1-HMMA	-81.5 ± 33.7
E2-HMMA	-76.7 ± 15.0
DHMA	95.4 ± 10.1
Caffeine	57.3 ± 12.3
1,7-dimethylxanthine	59.3 ± 9.4
Nicotine	-9.4 ± 7.1
Cotinine	49.0 ± 10.9
Creatinine	70.1 ± 2.3
Codeine	-5.2 ± 7.2
Oxycodone	-58.5 ± 11.5
Noroxycodone	-58.6 ± 7.8
Hydrocodone	-50.8 ± 7.6
Oxymorphone	-74.7 ± 10.5
Dihydrocodeine	-6.6 ± 11.6
Methadone	37.6 ± 19.9
EDDP	23.9 ± 1.8
E1-Venlafaxine	-19.3 ± 4.8
E2-Venlafaxine	-12.1 ± 9.5
Vardenafil	15.7 ± 10.8
E1-Norephedrine	63.4 ± 2.8
E2-Norephedrine	21.5 ± 4.6
E1-PMA	-21.7 ± 7.9
E2-PMA	-38.8 ± 4.1
Normorphine	63.2 ± 11.0
Dihydromorphine	60.0 ± 10.2

D1-Tramadol	22.1 ± 1.5
D2-Tramadol	8.8 ± 6.6
O-desmethyltramadol	46.7 ± 3.0
Zolpidem	-72.1 ± 3.2
Amitriptyline	-23.5 ± 0.6
Norketamine	-52.1 ± 2.4
Sildenafil	-49.9 ± 11.8
(+)-Ephedrine	-78.2 ± 4.6
(-)-Ephedrine and (-)-Ψephedrine	-72.3 ± 7.5
(+)-Ψephedrine	-76.7 ± 16.3
Desmethylvenlafaxine-E1	-6.3 ± 2.0
Desmethylvenlafaxine-E2	-31.1 ± 16.4
E1-Zopiclone	-33.2 ± 5.6
E2-Zopiclone	-41.0 ± 4.5
S-(+)-Fluoxetine	1.5 ± 0.1
R-(-)-Fluoxetine	3.2 ± 2.5
E1-Norfluoxetine	-4.3 ± 0.7
E2-Norfluoxetine	-11.0 ± 0.8

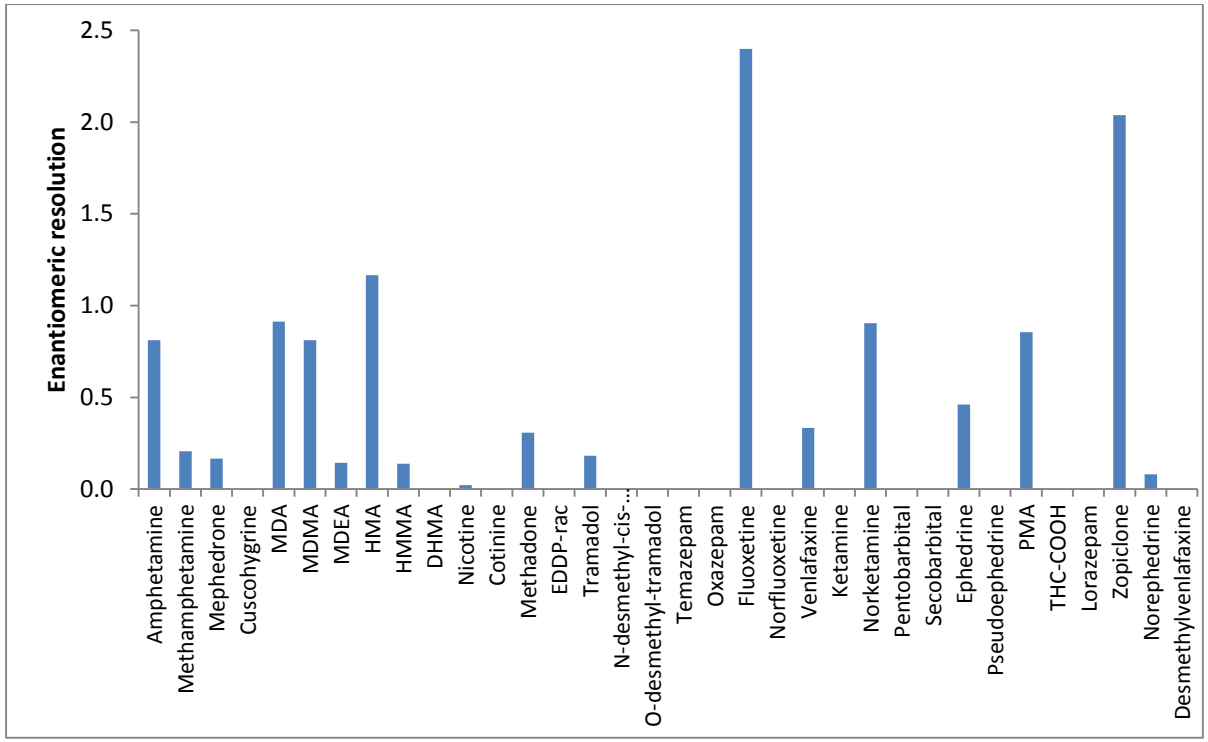


Figure S1 CBH column - **e**nantiomeric resolution of studied analytes in a mobile phase containing acetonitrile as organic modifier (mobile phase composition: 1mM ammonium acetate/acetonitrile 9:1).

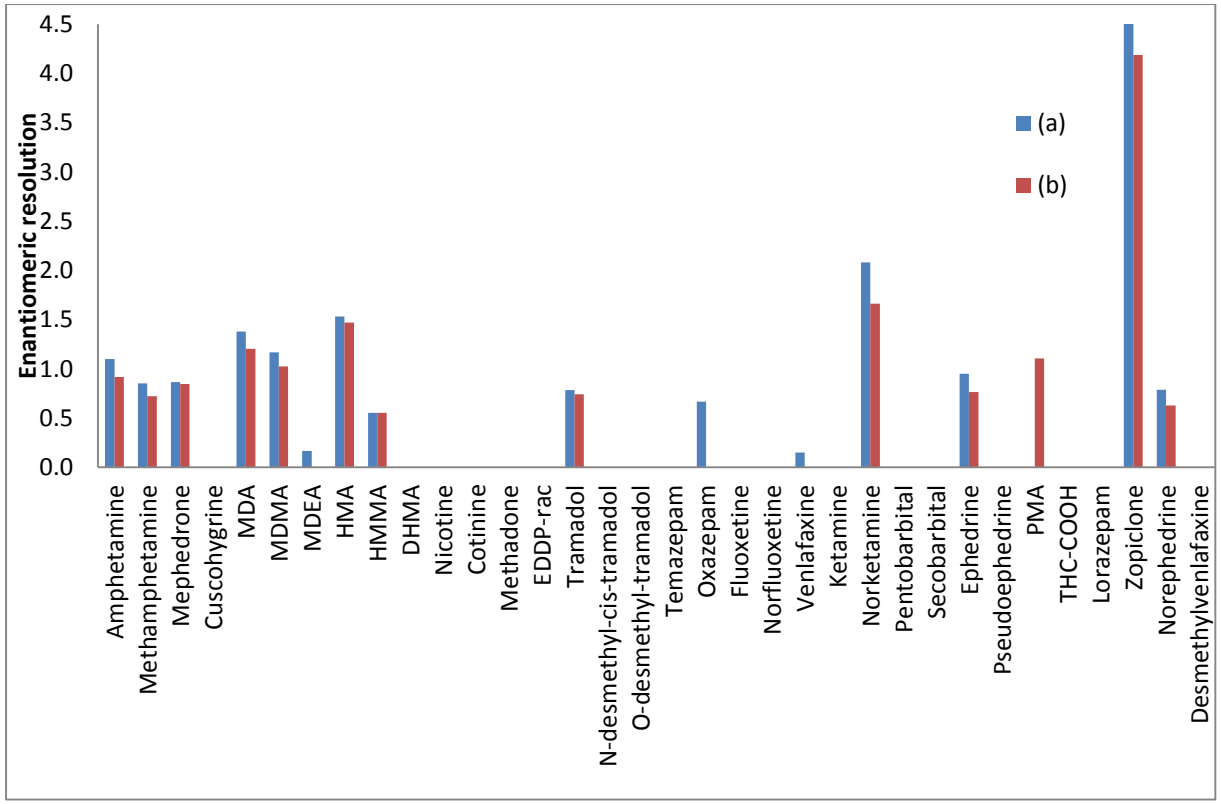


Figure S2 CBH column - **e**nantiomeric resolution of studied analytes in a mobile phase containing isopropanol as organic modifier (mobile phase composition: (a) 1mM ammonium acetate/isopropanol 9:1 and (b) 1mM ammonium acetate/isopropanol 9.5:0.5).

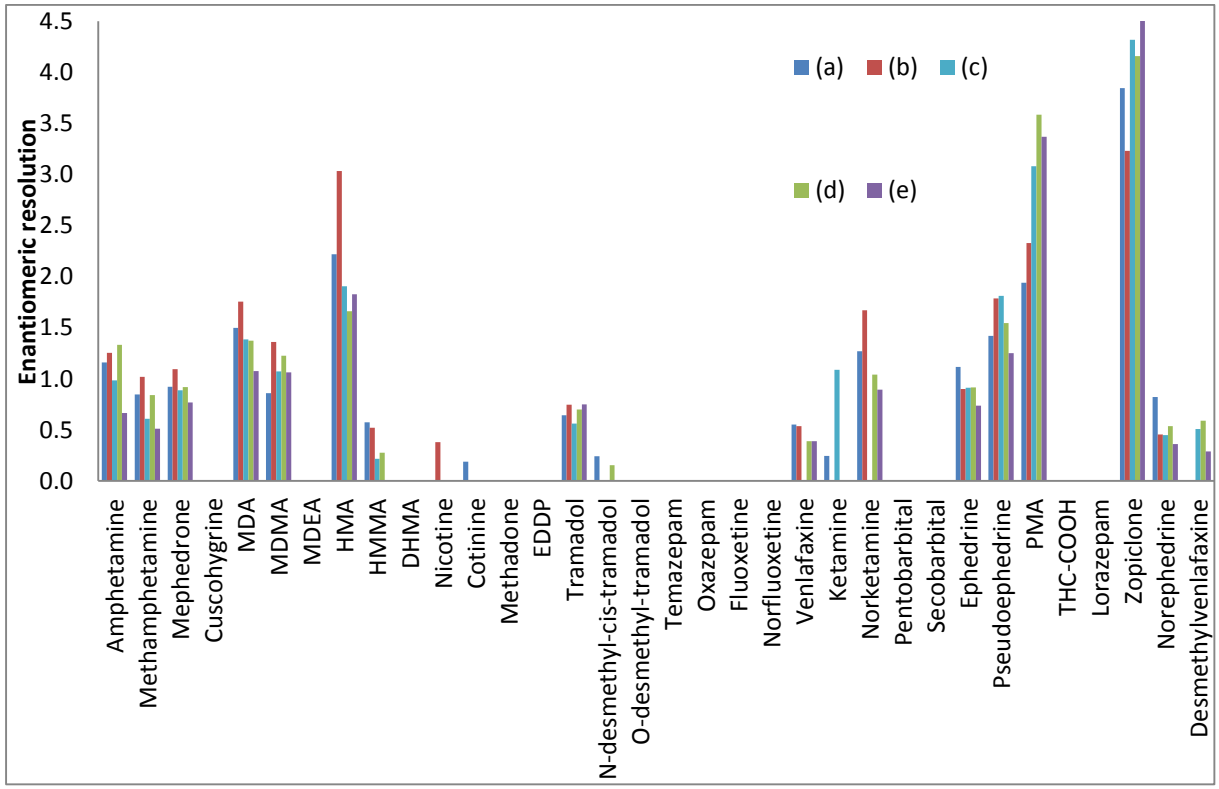
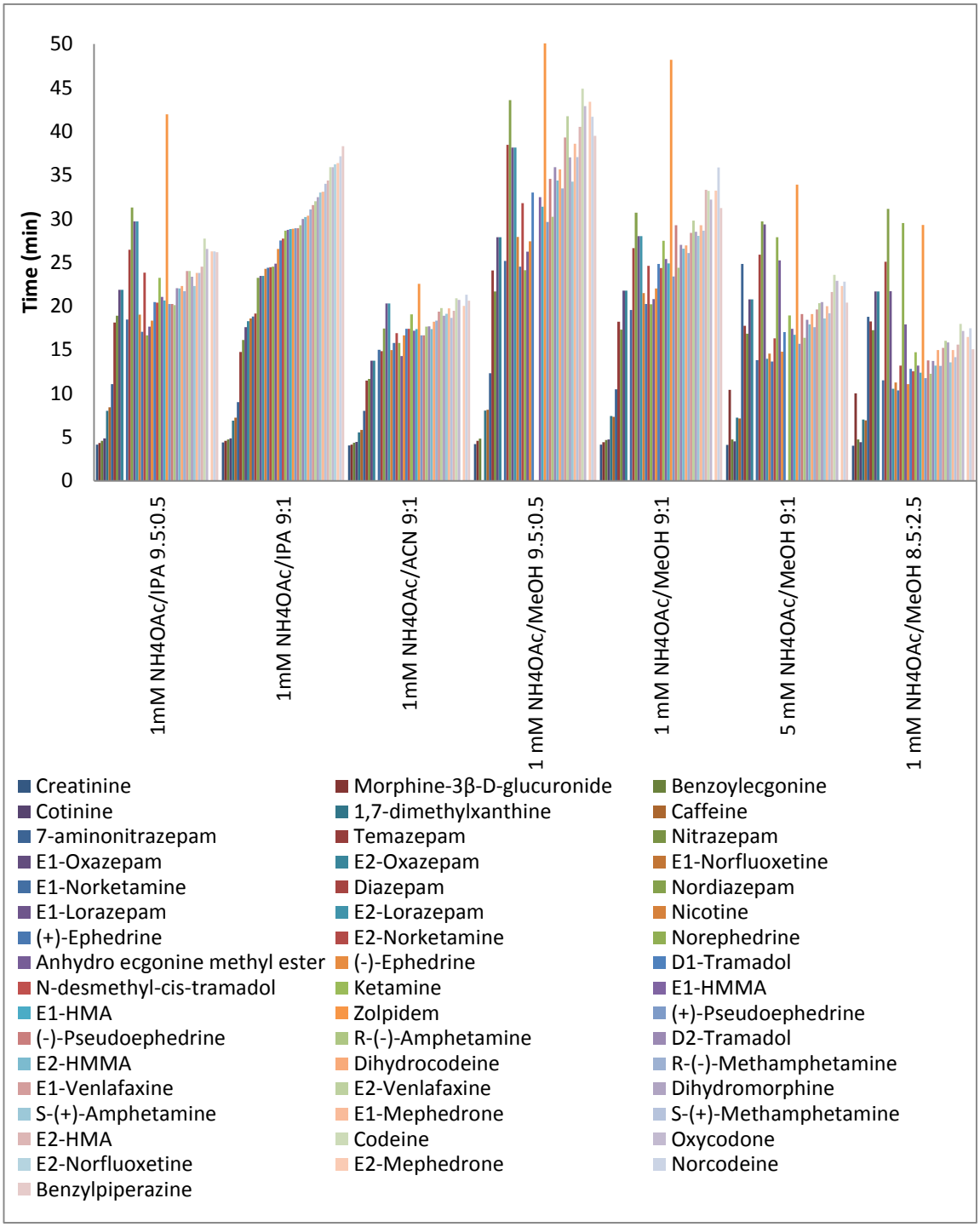


Figure S3 CBH column - enantiomeric resolution of studied analytes in mobile phases containing: (a) 1 mM ammonium acetate/methanol 9.5:0.5, (b) 1 mM ammonium acetate/methanol 9:1, (c) 2.5 mM ammonium acetate/methanol 9:1, (d) 5 mM ammonium acetate /methanol 9:1 and (e) 10 mM ammonium acetate /methanol 9:1.


Time (min)
50
45
40
35
30
25
20
15
10
5
0
1mM NH4OAc/IPA 9.5:0.5
1mM NH4OAc/IPA 9:1
1mM NH4OAc/ACN 9:1
1 mM NH4OAc/MeOH 9.5:0.5
1 mM NH4OAc/MeOH 9:1
5 mM NH4OAc/MeOH 9:1
1 mM NH4OAc/MeOH 8.5:2.5
Creatinine
Cotinine
7-aminonitrazepam
E1-Oxazepam
E1-Norketamine
E1-Lorazepam
(+)-Ephedrine
Anhydro ecgonine methyl ester
N-desmethyl-cis-tramadol
E1-HMA
(-)-Pseudoephedrine
E2-HMMA
E1-Venlafaxine
S-(+)-Amphetamine
E2-HMA
E2-Norfluoxetine
Benzylpiperazine
Morphine-3β-D-glucuronide
1,7-dimethylxanthine
Temazepam
E2-Oxazepam
Diazepam
E2-Lorazepam
E2-Norketamine
(-)-Ephedrine
Ketamine
Zolpidem
R-(-)-Amphetamine
Dihydrocodeine
E2-Venlafaxine
E1-Mephedrone
Codeine
E2-Mephedrone
Benzoylecgonine
Caffeine
Nitrazepam
E1-Norfluoxetine
Nordiazepam
Nicotine
Norephedrine
D1-Tramadol
E1-HMMA
(+)-Pseudoephedrine
D2-Tramadol
R-(-)-Methamphetamine
Dihydromorphine
S-(+)-Methamphetamine
Oxycodone
Norcodeine

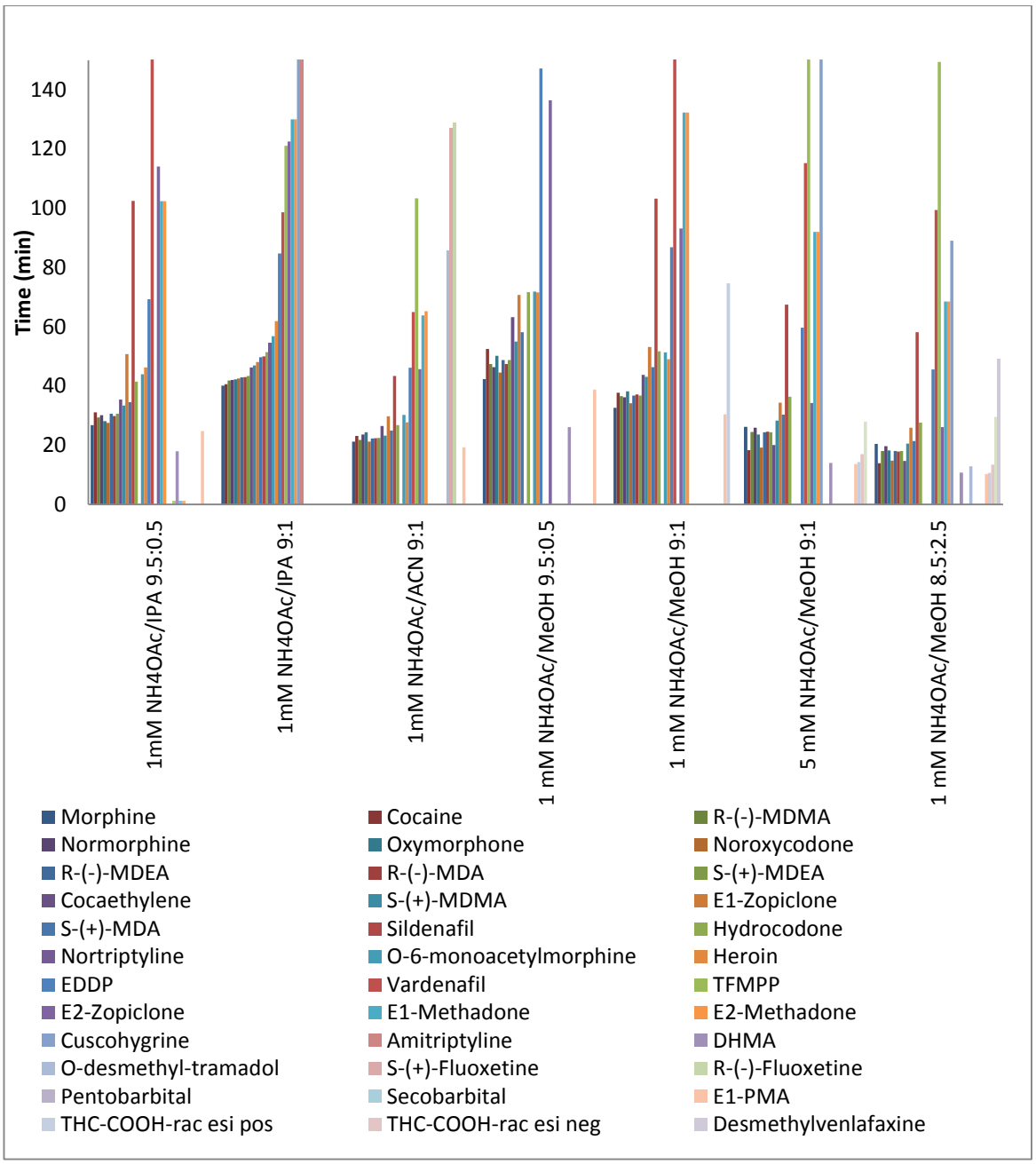


Figure S4 CBH column - Impact of different percentanges of modifiers on retention time of analytes (NH_4OAc: ammonium acetate, IPA: isopropanol, ACN: acetonitrile, MeOH: methanol).

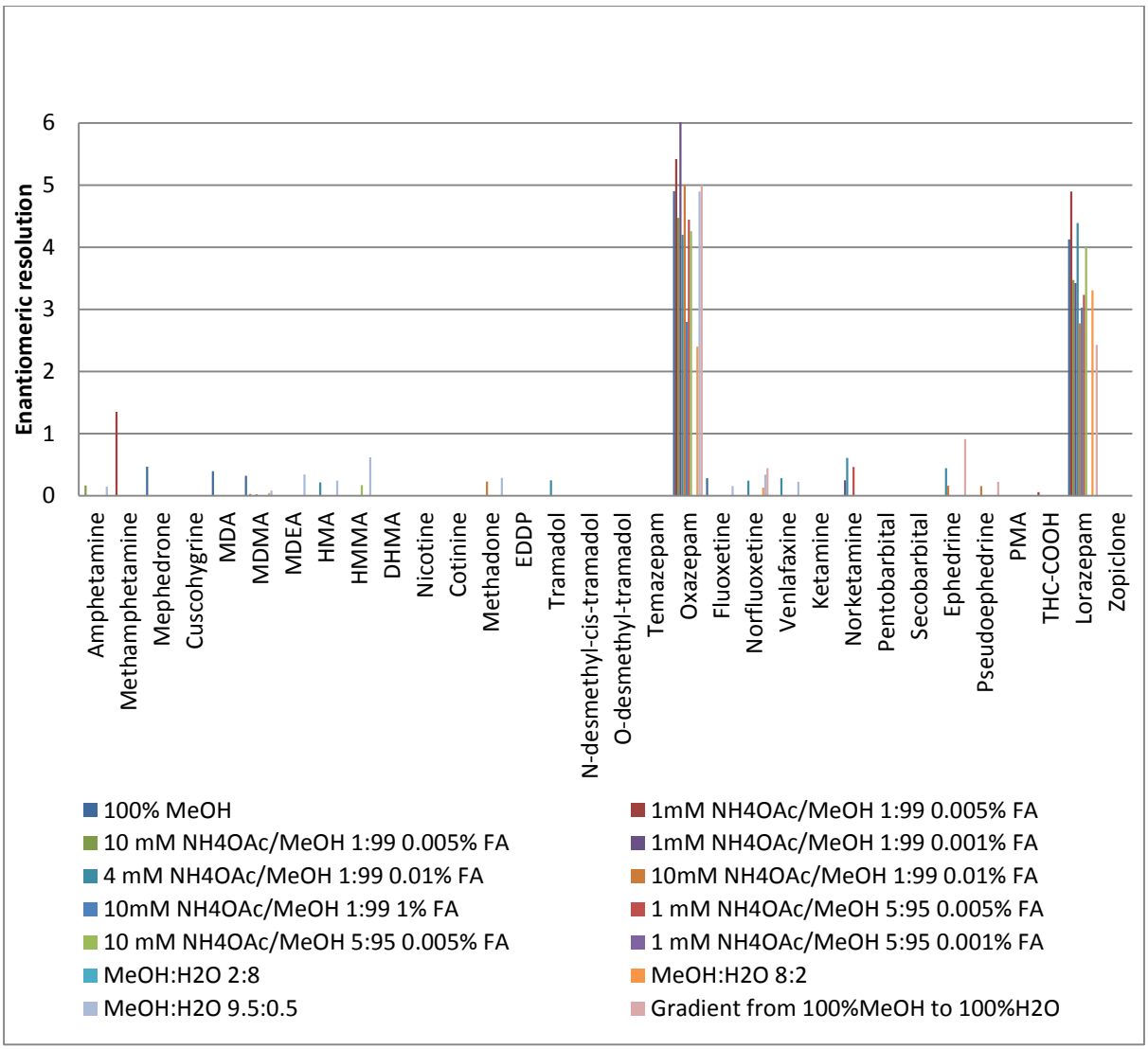


Figure S5 Chirobiotic T column - overview of the separation for the targeted analytes (MeOH: methanol, NH_4OAc: ammonium acetate, FA: formic acid).

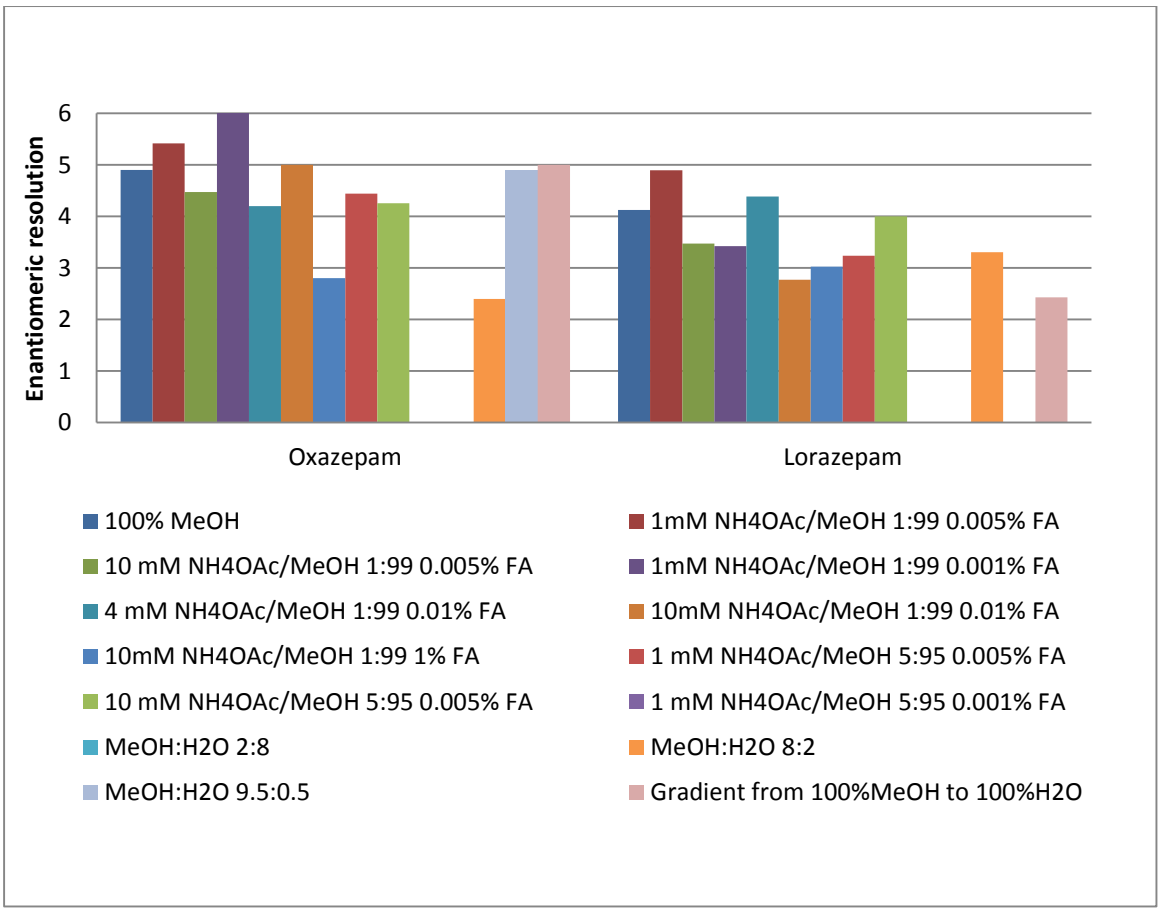


Figure S6 Chirobiotic T column - separation of oxazepam and lorazepam (MeOH: methanol, NH_4OAc: ammonium acetate, FA: formic acid.